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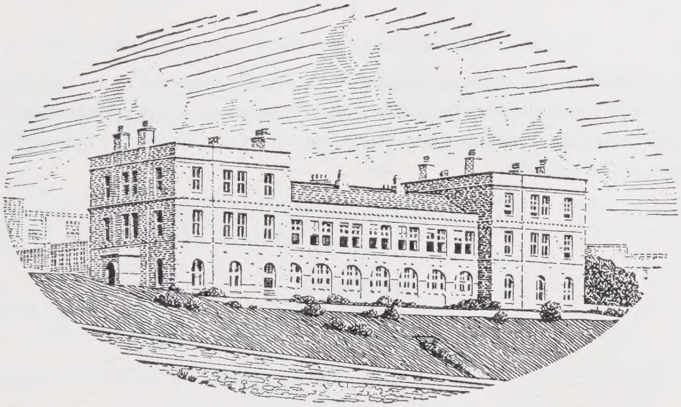

THE PLYMOUTH LABORATORY

VOLUME 37

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CONTENTS OF VOLUME 37

Number 1, pp. 1–266, February 1958
Number 2, pp. 267–534, June 1958
Number 3, pp. 535–870, October 1958

PAGE

Report of the Council:
1957–58 811

Income and Expenditure Account and Balance Sheet:
1957–58 835

List of Governors, Founders, Members, Honorary and Associate Members . . 839

Allen, J. A.
Observations on *Cochlodesma praetenue* (Pulteney) (Eulamellibranchia) 97

Alexandrowicz, J. S.
Further observations on proprioceptors in Crustacea and a hypothesis about their function 379

Armstrong, F. A. J.
Phosphorus and silicon in sea water off Plymouth during 1956 371
See also Wilson, Douglas P. and Armstrong, F. A. J. 331

Bagenal, T. B.
The fecundity of Clyde plaice 309

Bainbridge, V.
Some observations on *Evadne nordmanni* Lovén 349

Barnes, H.
The growth rate of *Verruca stroemia* (O. Müller) 427

Barrington, E. J. W.
The localization of organically bound iodine in the endostyle of *Amphioxus* . . 117

Burrows, Elsie M.
Sublittoral algal population in Port Erin Bay, Isle of Man 687

Clarke, B.
See Parke, Mary, Manton, Irene and Clarke, B. 209

Coles, John W.
Nematodes parasitic on sea weeds of the genera *Ascophyllum* and *Fucus* . . . 145

Cooper, L. N. H.
Sea temperatures in Plymouth Sound 1

Corner, E. D. S. and Rigler, F. H.
The modes of action of toxic agents. III. Mercuric chloride and *n*-amylmercuric chloride on crustaceans 85

Crisp, D. J.
The spread of *Elminius modestus* Darwin in north-west Europe 483

PAGE

CRISP, D. J. and SOUTHWARD, A. J.
The distribution of intertidal organisms along the coasts of the English Channel . 157

CULKIN, F. and RILEY, J. P.
The occurrence of gallium in marine organisms 607

DALES, R. PHILLIPS
Survival of anaerobic periods by two intertidal polychaetes, *Arenicola marina* (L.) and *Owenia fusiformis* Delle Chiaje 521
See also KENNEDY, G. Y. and DALES, R. PHILLIPS 15

DAISLEY, K. W.
A method for the measurement of vitamin B_{12} concentration in sea water . . 673

DAISLEY, K. W. and FISHER, L. R.
Vertical distribution of vitamin B_{12} in the sea 683

DENTON, E. J. and MARSHALL, N. B.
The buoyancy of bathypelagic fishes without a gas-filled swimbladder . . . 753

DROOP, M. R.
Requirement for thiamine among some marine and supra-littoral protista . . 323

FOGG, G. E.
See KAIN, JOANNA M. and FOGG, G. E. 397
See KAIN, JOANNA M. and FOGG, G. E. 781

FISHER, L. R.
See DAISLEY, K. W. and FISHER, L. R. 683

FORSTER, G. R.
Underwater observations on the fauna of shallow rocky areas in the neighbourhood of Plymouth 473

FREEMAN, R. F. H. and LLEWELLYN, J.
An adult digenetic trematode from an invertebrate host: *Proctoeces subtenuis* (Linton) from the lamellibranch *Scrobicularia plana* (da Costa) 435

GREEN, J.
Eudactylina rachelae n.sp., a copepod parasitic on the electric ray, *Torpedo nobiliana* Bonaparte 113

HANCOCK, D. A.
Notes on starfish on an Essex oyster bed 565

HEDLEY, R. H.
Tube formation by *Pomatoceros triqueter* (Polychaeta) 315

KAIN, JOANNA M.
Observations on the littoral algae of the Isle of Wight 769

KAIN, JOANNA M. and FOGG, G. E.
Studies on the growth of marine phytoplankton. I. *Asterionella japonica* Gran . 397
Studies on the growth of marine phytoplankton. II. *Isochrysis galbana* Parke . 781

KENNEDY, G. Y. and DALES, R. PHILLIPS.
The function of the heart-body in polychaetes 15

PAGE

LLEWELLYN, J.
The adhesive mechanisms of monogenetic trematodes: the attachment of species of the Diclidophoridae to the gills of gadoid fishes 67

LLEWELLYN, J.
See FREEMAN, R. F. H. and LLEWELLYN, J. 435

MANTON, IRENE
See PARKE, MARY, MANTON, IRENE and CLARKE, B. 209

MARSHALL, N. B.
See DENTON, E. J. and MARSHALL, N. B. 753

MARSHALL, S. M. and ORR, A. P.
On the biology of *Calanus finmarchicus*. X. Seasonal changes in oxygen consumption 459

MASON, JAMES
The breeding of the scallop, *Pecten maximus* (L.), in Manx waters 653

MILLAR, R. H.
The breeding season of some littoral ascidians in Scottish waters 649

MORTON, J. E.
Observations on the gymnosomatous pteropod *Clione limacina* (Phipps) . . . 287

MURPHY, J. and RILEY, J. P.
A single-solution method for the determination of soluble phosphate in sea water . 9

NEEDLER, MARY and ROSS, D. M.
Neuromuscular activity in sea anemone *Calliactis parasitica* (Couch) 789

NEWELL, G. E.
The behaviour of *Littorina littorea* (L.) under natural conditions and its relation to position on the shore 229
An experimental analysis of the behaviour of *Littorina littorea* (L.) under natural conditions and in the laboratory 241

NICOL, J. A. C.
Luminescence in polynoids. IV. Measurements of light intensity 33
Spectral composition of the light of *Pholas dactylus* L. 43
Observations on luminiscence in *Noctiluca* 535
Observations on the luminescence of *Pennatula phosphorea*, with a note on the luminiscence of *Virgularia mirabilis* 551
Observations on luminescence in pelagic animals 705

ORR, A. P.
See MARSHALL, S. M. and ORR, A. P. 459

PARKE, MARY, MANTON, IRENE and CLARKE, B.
Studies on marine flagellates. IV. Morphology and microanatomy of a new species of *Chrysochromulina* 209

RIGLER, F. H.
See CORNER, E. D. S. and RIGLER, F. H. 85

PAGE

RILEY, J. P.
See CULKIN, F. and RILEY, J. P 607
See MURPHY, J. and RILEY, J. P. 9

ROSS, D. M.
See NEEDLER, MARY and ROSS, D. M. 789

RUSSELL, F. S.
Notes on the medusa *Amphinema krampi* Russell 81

SOUTHWARD, A. J.
Note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution 49
See also
CRISP, D. J. and SOUTHWARD, A. J. 157
SOUTHWARD, EVE C. and SOUTHWARD, A. J. 267, 627

SOUTHWARD, A. J. and SOUTHWARD, EVE C.
On the occurrence and behaviour of two little-known barnacles, *Hexelasma hirsutum* and *Verruca recta*, from the continental slope 633

SOUTHWARD, EVE C. and SOUTHWARD, A. J.
The breeding of *Arenicola ecaudata* Johnston and *A. branchialis* Aud. & Edw. at Plymouth 267
On some Pogonophora from the north-east Atlantic, including two new species . 627
See also SOUTHWARD, A. J. and SOUTHWARD, EVE C. 633

SPENCER, C. P.
The chemistry of ethylenediamine tetra-acetic acid in sea water 127

THOMAS, H. J.
Observations on the increase in size at moulting in the lobster (*Homarus vulgaris* M.-Edw.) 603

WALNE, P. R.
The importance of bacteria in laboratory experiments on rearing the larvae of *Ostrea edulis* (L.) 415

WALNE, P. R.
Growth of oysters (*Ostrea edulis* L.) 591

WILSON, DOUGLAS P.
On some small *Iathina janthina* (L.) stranded on the Isles of Scilly, 1957 . . . 5
Notes from the Plymouth Aquarium. III. 299
The polychaete *Magelona alleni* n.sp., and a reassessment of *Magelona cincta* Ehlers 617

WILSON, DOUGLAS P. and ARMSTRONG, F. A. J.
Biological differences between sea waters: experiments in 1954 and 1955 . . 331

ABSTRACTS OF MEMOIRS
RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

PAGE

The positive and negative heat production associated with a nerve impulse. By B. C. Abbott, A. V. Hill and J. V. Howarth 807

Contraction in molluscan smooth muscle. By B. C. Abbott and J. Lowy . . 807

Mechanical properties of *Helix* and *Mytilus* muscle. By B. C. Abbott and J. Lowy . 808

Observations on the distribution of amine oxidase in invertebrates. By H. Blaschko and D. B. Hope 531

Niobium in ascidians. By D. B. Carlisle 808

Niobium in sea-water. By D. B. Carlisle and L. G. Hummerstone . . . 808

The spectral variation of the photosensitivities of some visual pigments. By H. J. A. Dartnall 531

The visual pigment of the conger eel. By E. J. Denton & M. A. Walker . . . 808

Structure of smooth muscles. By Jean Hanson and J. Lowy 531

The annelid phosphokinases. By G. E. Hobson and K. R. Rees 532

The contractility and healing behaviour of pieces of *Leucosolenia complicata*. By W. C. Jones 532

Observations on photophores and luminescence in the teleost *Porichthys*. By J. A. C. Nicol 532

Sensitivity to light in the sea-anemone *Metridium senile* (L.): adaption and action spectra. By W. J. North and C. F. A. Pantin 809

The structure and hydromechanics of the musculo-epithelium in *Metridium*. By Elaine A. Robson 809

Quick and slow contractions in the isolated sphincter of the sea anemone, *Calliactis parasitica*. By D. M. Ross 532

Observations on the food canal and associated structures in the holothurian *Holothuria forskali* delle Chiaje. By F. C. Stott 533

The feeding mechanism, functional morphology and histology of the alimentary canal of *Terebella lapidaria* L. (Polychaeta). By Muriel F. Sutton 809

CORRIGENDA

Vol. 37

Page 39, line 19; page 741, line 9: for *atlantica* read *atlanticum*

Page 159, Table 1, first column, line 15; page 180, bottom side heading; page 182, line 18; page 198, line 19: for *polysichides* read *polyschides.*

Page 172, line 13 *for* Halford *read* Helford

Page 627, Table 1, last column; p. 630, heading; p. 631, fig. 3 legend; p. 632, lines 4 and 11: for *inermis* read *inerme.*

Page 718, line 15, *for* peduncle *read* pedalium.

Page 723, line 5 for *Plesiopennaeus* read *Plesiopenaeus.*

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SEA TEMPERATURES IN PLYMOUTH SOUND

By L. H. N. Cooper, D.Sc.
The Plymouth Laboratory

For ecological studies records of mean sea temperatures in and near Plymouth Sound are often required. The necessary data exist but have not been readily available in a convenient form.

Since 1898 sea temperature has been included amongst the observations made by the Plymouth City Meteorologist. In early days this was Mr Victor Prigg whose successors, Mr G. H. Ivory and Messrs G. H. Ivory and Partners continued the work until 1954. In that year the work was transferred to the Department of the Medical Officer of Health for Plymouth. The measurements on the Fahrenheit scale appear always to have been made at a depth of 1 fathom (1·8 m) at about the time of high water. They were made three times a week from the end of the Promenade Pier until it was destroyed by fire in March 1941 and afterwards from the end of the 'Banjo Pier' until 1955. For the last two years they have been taken from the Pier Head of Millbay Docks, 0·3 nautical mile (0·55 km) distant. The three positions have a very similar exposure to the tidal stream and the observers by a series of comparable observations assured themselves that no appreciable error was introduced by the changes.

Five-year running means (anomalies in ° C) for air temperatures on Plymouth Hoe have been published by Southward & Crisp (1954). In half a century they show a rise of more than 0·5° C. Monthly means of many meteorological measurements on Plymouth Hoe have been published for many years in the *Transactions of the Devonshire Association*, but sea temperatures have not been included. Annual means of sea temperature have appeared somewhat erratically in the Annual Reports of the Medical Officer of Health. Monthly means, although prepared, have never been published except for the 8 years 1912–19 (Orton, 1920).

Mr E. Voaden of Messrs G. H. Ivory and Partners and Mr D. W. A. Cole of the Department of the Medical Officer of Health have generously provided us with the monthly means for each month from January 1947 to December 1956. Converted to the Centigrade scale they are printed here as Table 1. For each month the 10-year mean has been computed. Monthly means averaged over the 55 years 1898–1953 have also been provided by Mr Voaden. To facilitate future computing, they are reported on both scales of temperature (Table 2). During the years 1912–19 the months January–September were colder and the months October–December were markedly warmer than the 55-year mean. In the years 1947–56, ten of the monthly means are well above

TABLE 1. MONTHLY MEAN SEA TEMPERATURES (° C) FOR PLYMOUTH SOUND 1947–1956 TOGETHER WITH MAXIMA AND MINIMA ATTAINED IN EACH MONTH

	Jan.			Feb.			Mar.			Apr.			May			June		
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
1947	8·3	9·1	6·2	5·3	6·5	4·3	6·4	8·3	4·7	9·6	11·6	8·0	11·2	14·1	8·4	13·6	15·3	12·2
1948	9·5	10·0	8·6	8·6	9·4	6·9	8·4	9·1	7·4	9·9	11·1	8·9	12·3	14·2	10·8	14·0	14·1	12·8
1949	9·5	9·9	8·8	9·3	9·7	8·2	9·2	10·1	8·8	10·4	11·3	9·3	12·1	13·2	10·7	14·6	17·1	13·0
1950	9·4	10·2	7·6	8·4	9·1	7·8	9·3	10·1	7·8	10·0	10·6	9·4	12·2	14·2	11·0	15·1	16·1	14·2
1951	8·2	8·9	7·7	7·9	8·2	7·6	8·1	8·3	7·6	8·9	9·9	8·2	10·7	12·2	9·5	13·7	14·7	12·3
1952	9·0	9·8	8·1	8·2	8·7	7·7	8·9	9·8	7·8	9·7	10·8	7·8	12·5	13·8	10·9	14·2	15·3	13·7
1953	8·5	8·9	7·9	7·8	8·6	7·1	8·4	9·6	8·0	9·6	10·3	8·9	11·9	13·2	10·3	13·2	14·6	11·4
1954	8·7	11·4	7·9	7·5	8·3	6·5	8·6	9·6	7·7	10·0	11·5	9·1	11·5	12·9	9·9	13·7	14·7	12·6
1955	8·6	9·4	7·3	7·9	8·9	6·7	6·9	7·8	6·3	8·6	9·5	7·2	10·2	11·8	9·4	13·2	15·0	12·2
1956	9·8	10·1	9·3	7·7	9·4	6·1	7·9	8·7	7·2	9·3	9·7	8·7	11·3	12·2	9·7	12·5	13·4	11·6
10-year average	8·95	—	—	7·86	—	—	8·21	—	—	9·60	—	—	11·59	—	—	13·78	—	—

	July			Aug.			Sept.			Oct.			Nov.			Dec.			
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Annual mean
1947	14·9	17·1	13·4	17·6	18·6	16·1	17·2	18·3	15·7	15·2	15·6	13·5	13·1	13·7	12·1	10·9	12·2	9·7	11·9
1948	14·7	17·0	13·3	16·0	16·9	15·3	15·4	16·7	14·7	14·3	15·4	12·7	12·3	13·0	11·3	10·7	11·9	9·0	12·2
1949	17·3	18·3	16·3	17·0	18·3	16·3	17·5	18·9	16·9	16·1	17·2	12·8	12·6	14·1	11·6	9·5	11·7	9·4	12·9
1950	15·7	16·6	14·6	16·4	17·1	15·4	15·7	16·1	14·2	13·8	14·6	12·4	11·5	12·4	10·6	9·3	10·8	7·6	12·2
1951	15·2	16·4	14·7	15·9	17·1	15·2	15·2	15·8	14·8	14·1	14·9	12·8	12·2	12·8	11·6	10·6	11·7	8·9	11·7
1952	15·7	16·7	14·9	16·4	17·2	15·6	15·2	16·9	14·3	13·4	14·3	12·9	11·6	13·2	9·4	9·1	9·4	8·7	12·0
1953	15·0	15·7	13·9	16·1	17·6	13·9	15·4	16·1	14·8	14·4	15·5	13·5	12·6	13·1	12·2	12·0	12·7	11·1	12·1
1954	13·8	14·3	13·3	14·4	15·5	13·7	15·2	16·8	13·9	14·2	14·8	13·6	12·1	13·2	10·8	10·9	11·8	9·7	11·7
1955	15·7	17·5	14·0	17·4	18·9	16·1	16·4	18·3	15·1	14·4	15·6	12·8	12·3	13·3	11·5	11·1	11·6	10·6	11·9
1956	14·6	15·6	13·7	15·1	15·5	14·4	15·0	15·6	14·2	14·4	15·4	13·3	12·3	13·3	11·2	10·6	11·3	9·4	11·8
10-year average	15·27	—	—	16·22	—	—	15·81	—	—	14·43	—	—	12·26	—	—	10·47	—	—	12·04

TABLE 2. AVERAGE MEAN MONTHLY TEMPERATURES (° C) OVER PERIODS OF YEARS

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
55-year average, ° F, 1898–1952	47·9	46·5	46·9	49·0	52·5	56·8	59·6	60·9	60·1	57·0	53·2	49·9
55-year average, ° C, 1898–1952	8·8$_3$	8·0$_6$	8·2$_8$	9·4$_4$	11·3$_9$	13·7$_8$	15·3$_3$	16·0$_6$	15·6$_1$	13·8$_9$	11·7$_8$	9·9$_4$
8-year average, ° C, 1912–19	8·6$_1$	7·8$_3$	7·9$_4$	9·0$_0$	11·2$_2$	13·4$_1$	15·0$_0$	15·9$_7$	15·5$_3$	14·0$_0$	12·0$_0$	10·3$_3$
10-year average, ° C, 1947–56	8·9$_5$	7·8$_6$	8·2$_1$	9·6$_0$	11·5$_9$	13·7$_8$	15·2$_7$	16·2$_2$	15·8$_1$	14·4$_3$	12·2$_6$	10·4$_7$
Difference, ° C, 1912–19 from 55-year average	−0·2$_2$	−0·2$_3$	−0·3$_4$	−0·4$_4$	−0·1$_7$	−0·3$_4$	−0·3$_3$	−0·0$_9$	−0·0$_8$	+0·1$_1$	+0·2$_2$	+0·3$_9$
Difference, ° C, 1947–56 from 55-year average	+0·1$_2$	−0·2$_0$	−0·0$_7$	+0·1$_6$	+0·2$_0$	0·0$_0$	−0·0$_6$	+0·1$_6$	+0·2$_0$	+0·5$_4$	+0·4$_8$	+0·5$_3$
Difference, ° C, 1947–56 from 1912 to 1919	+0·3$_4$	+0·0$_3$	+0·2$_7$	+0·6$_0$	+0·3$_7$	+0·3$_4$	+0·2$_7$	+0·2$_5$	+0·2$_8$	+0·4$_0$	+0·2$_6$	+0·1$_4$

the long-term average. If the exceptionally cold months of February and March 1947 are omitted the monthly means for February and March for the remaining nine years were both also warmer than average. The years 1947–56 (even with February and March 1947 included) were on average warmer in every month than the years 1912–19. This trend is now well known to climatologists.

Atkins & Jenkins (1952) have published monthly means for International Hydrographic Station E1 (50° 02′ N., 4° 22′ W.) and drew attention to some exceptional years. They presented no evidence for long-term changes. From the same observations I also sought evidence for long-term changes and failed to find it. The cruises are made about once a month, and there are inevitably many sources of bias in the raw data. A systematic study of the E1 records which will stand statistical criticism will be tedious but needs to be made. Until this is done, the thrice-weekly records from Plymouth Sound provide a better basis for long-term ecological comparisons than do the monthly records from Station E1.

Of recent years there are indications that the composition of the fauna of the English Channel has changed due to an increase in the occurrence of southern, warmer-water forms. The rise of about 0·3° C, found in Plymouth Sound, may well be a sufficient explanation: if it is, there is no point in seeking an explanation in other terms (as, for example, Cooper, 1955). I prefer to believe for the present that we have here interlocking facets of a global change and that the simple explanation by itself is not sufficient.

SUMMARY

Monthly mean sea-water temperatures in Plymouth Sound, prepared by the Plymouth City Meteorologist, are presented as data for ecological studies in this laboratory. In the last 40 years there has been a rise in mean temperature of about 0·3° C.

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ON SOME SMALL *IANTHINA JANTHINA* (L.) STRANDED ON THE ISLES OF SCILLY, 1957

By DOUGLAS P. WILSON, D.Sc.
The Plymouth Laboratory

(Text-fig. 1)

In an earlier paper (Wilson & Wilson, 1956) there was described a large-scale stranding, on the shores of Cornwall and Devon in 1954, of the pelagic gastropod *Ianthina janthina* (L.). Most of the shells then collected were broken, but thirty-six were entire and measurements of these showed that, in spite of considerable variation in the proportion of width to height, the width is proportionately greater in young than in old shells. None of the 1954 shells was less than half an inch high, the majority being considerably higher. Two shells stranded at Sennen several years before, and the figure of one in a paper by Fowler (1947, as *I. planispirata* Adams & Reeve) were the only specimens less than half-an-inch high available for comparison with the larger ones. The measurements of these agreed with the general conclusion that as the shell grows it becomes proportionately less wide. In the past these variations in shell shape have been responsible for the erection of a number of different species. In order to establish the conclusion firmly it was desirable to examine a collection of young shells.

On 31 March 1957 Mrs M. Hicks wrote from St Agnes, Isles of Scilly, to say that on that day her husband had found eight small *Ianthina*, six of which had stranded alive on the afternoon's tide, the other two, one of them alive, earlier. Subsequently more were collected. On 8 April Miss Deborah Hicks (age 9) picked up about 130, some of them living, some apparently not. On 10 April about thirty-two more in the same general condition were obtained, and on 11 April another twenty-four, all apparently dead.

When examined in Plymouth all these specimens proved to be small *I. janthina* (L.), mostly of a shape formerly described as *I. planispirata* Adams & Reeve. Almost all were less than half an inch high, being generally much smaller, and most of the shells were unbroken. Such a large collection of small shells of this species probably does not exist elsewhere, and it is therefore of interest to record their measurements and to compare them with the lesser collection of large shells described last year.

As before, the shells were measured with calipers graduated in hundredths of an inch. These measurements are recorded in Table 1; the width/height ratios were again worked out. In the graph in Fig. 1 the ratio for each shell is

TABLE 1. MEASUREMENT OF SHELLS OF *IANTHINA JANTHINA* (L.)

No. of shells	Totals: each height	Height (in.)	Width (in.)	Ratio: width/height
1	1	0·22	0·28	1·27
2	5	0·23	0·30	1·30
2		0·23	0·31	1·35
1		0·23	0·32	1·39
1	5	0·24	0·31	1·29
1		0·24	0·33	1·38
3		0·24	0·34	1·42
1	4	0·25	0·33	1·32
2		0·25	0·34	1·36
1		0·25	0·37	1·48
1	5	0·26	0·34	1·31
1		0·26	0·35	1·35
1		0·26	0·36	1·38
2		0·26	0·37	1·42
3	13	0·27	0·33	1·22
1		0·27	0·35	1·30
1		0·27	0·36	1·33
4		0·27	0·37	1·37
2		0·27	0·38	1·41
2		0·27	0·39	1·44
3	8	0·28	0·36	1·29
1		0·28	0·37	1·32
2		0·28	0·38	1·36
1		0·28	0·39	1·39
1		0·28	0·40	1·43
1	10	0·29	0·35	1·21
2		0·29	0·36	1·24
2		0·29	0·37	1·27
1		0·29	0·38	1·31
1		0·29	0·39	1·34
2		0·29	0·40	1·38
1		0·29	0·41	1·41
2	13	0·30	0·38	1·27
5		0·30	0·39	1·30
3		0·30	0·40	1·33
2		0·30	0·41	1·37
1		0·30	0·42	1·40
1	12	0·31	0·38	1·23
5		0·31	0·40	1·29
3		0·31	0·41	1·32
2		0·31	0·42	1·36
1		0·31	0·43	1·39
1	15	0·32	0·40	1·25
3		0·32	0·41	1·28
5		0·32	0·42	1·31
4		0·32	0·43	1·34
1		0·32	0·44	1·38
1		0·32	0·48	1·50
1	7	0·33	0·40	1·21
3		0·33	0·42	1·27
3		0·33	0·43	1·30
2	8	0·34	0·40	1·18
2		0·34	0·43	1·26
1		0·34	0·44	1·29
2		0·34	0·45	1·32
1		0·34	0·46	1·35
2	6	0·35	0·44	1·26
1		0·35	0·45	1·29
3		0·35	0·46	1·31
2	5	0·36	0·45	1·25
3		0·36	0·47	1·31
1	6	0·37	0·46	1·24
1		0·37	0·47	1·27
2		0·37	0·48	1·30
1		0·37	0·49	1·32
1		0·37	0·50	1·35
2	4	0·38	0·47	1·24
1		0·38	0·48	1·26
1		0·38	0·49	1·29
1	1	0·39	0·51	1·31
0	0	0·40	—	—
1	5	0·41	0·52	1·27
1		0·41	0·53	1·29
1		0·41	0·54	1·32
1		0·41	0·55	1·34
1		0·41	0·57	1·39
1	3	0·42	0·54	1·29
2		0·42	0·57	1·36
2	3	0·43	0·55	1·28
1		0·43	0·59	1·37
1	3	0·44	0·56	1·27
1		0·44	0·57	1·30
1		0·44	0·58	1·32
1	7	0·45	0·54	1·20
1		0·45	0·55	1·22
2		0·45	0·56	1·24
2		0·45	0·60	1·33
1		0·45	0·62	1·38
1	5	0·46	0·58	1·26
2		0·46	0·59	1·28
1		0·46	0·60	1·30
1	2	0·47	0·57	1·21
1		0·47	0·60	1·28
1	1	0·48	0·60	1·25
1	1	0·49	0·59	1·20
3	5	0·50	0·64	1·28
1		0·50	0·65	1·30
1		0·50	0·66	1·32
1	2	0·51	0·64	1·26
1		0·51	0·67	1·31
1	1	0·52	0·69	1·33

plotted against the height. This graph is identical with that in Wilson & Wilson (1956, text-fig. 2) with these shells added. It will be seen that their positions on the graph are in good agreement with the line which was originally based on the fewer and mainly larger shells measured previously. The shells marked I and II have not only wide apertures but also spires flatter than most. The shells marked III have average spires but their apertures, and hence cross-sections of the coil, are noticeably narrow compared with most others; this gives these shells a tall appearance.

It appears that the shells comprise two main size-groups. The first of these contains 128 shells ranging in height from 0·22 to 0·39 in. with a peak (fifteen shells) at 0·32 in. The second group contains thirty-seven shells ranging in height from 0·41 to 0·52 in. with a peak (seven shells) at 0·45 in. These two groups are obvious in the graph (Fig. 1) on either side of height

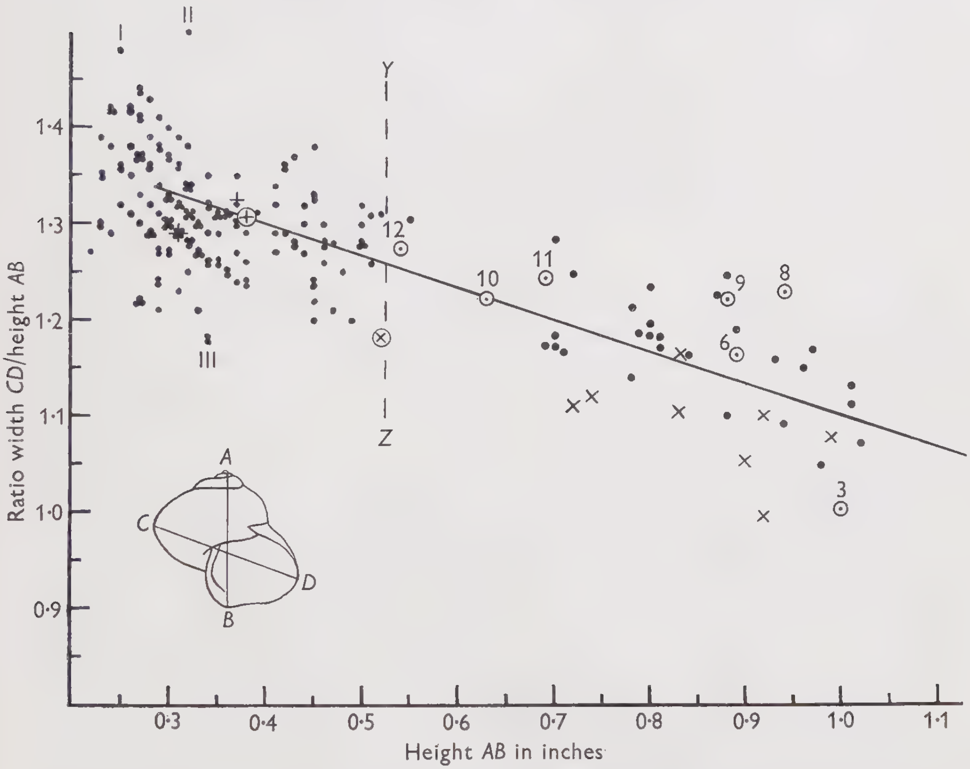

Fig. 1. Left-hand portion of the graph reproduced in Wilson & Wilson, 1956, p. 299, to which have been added, to the left of the broken line *YZ*, data for young *Ianthina janthina* shells stranded in the Isles of Scilly, March and April 1957. The graph shows the relation of shell width to shell height, expressed as ratio of width *CD* to height *AB*. Shells marked I–III are described in the text. For explanation of the remaining symbols see the legend to the original figure, reference as above.

0·40 in. There was no shell of that height in the collection. There is nothing to indicate what these groups represent, whether age, sex or other differences, but the presence of the peaks with almost regular falling off in numbers on either side (see Table 1) makes it likely that they are not purely fortuitous.

The new shells add appreciably to the data previously available, especially for small sizes, and confirm earlier conclusions concerning the effect of growth on the relations between width and height. It only remains to express grateful thanks to Mr and Mrs Hicks, and especially their daughter Deborah, for collecting the shells and forwarding them with such care.

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A SINGLE-SOLUTION METHOD FOR THE DETERMINATION OF SOLUBLE PHOSPHATE IN SEA WATER

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Of the reagents so far suggested for the reduction of phosphomolybdic acid to molybdenum blue in the determination of phosphate, only stannous chloride (Atkins, 1923; Harvey, 1948), metol (Burton & Riley, 1956), and ascorbic acid (Greenfield & Kalber, 1954), give colours of sufficient intensity to be used in the analysis of sea water.

Burton & Riley (1956) have recently reviewed the use of stannous chloride as a reducing agent in the molybdenum-blue method for the determination of phosphate in sea water. This reagent suffers from a number of disadvantages; in particular that (i) the time taken for development of maximum colour depends on the salinity of the water and its temperature, (ii) the colour fades quite rapidly, (iii) there is a large salt error, and (iv) different batches of molybdate reagent give different extinctions with the same amount of phosphorus.

These workers have proposed the use of metol (*p*-methyl-aminophenol sulphate) for the reduction of phosphomolybdic acid in the analysis of sea water. This reagent is free from most of the disadvantages of stannous chloride, but requires the development of colour to be carried out at 100° C.

The use of ascorbic acid for the reduction of phosphomolybdic acid was first reported by Ammon & Hinsberg (1936). Their method was modified by Lowry, Roberts, Leiner, Wu & Farr (1954), who used a greater concentration of ascorbic acid. Essentially the same procedure was employed by Chen, Toribara & Warner (1956) for the micro-determination of phosphorus in biological fluids. Greenfield & Kalber (1954) have suggested the use of ascorbic acid for the determination of phosphorus in sea water.

When this reductant was investigated, it was found possible to use a single reagent containing ammonium molybdate, ascorbic acid and sulphuric acid, instead of adding the acid molybdate and reducing agent separately, as in the stannous chloride method. The present paper describes the application of this mixed reagent to the determination of soluble phosphate in sea water.

The relative amounts of sulphuric acid and ammonium molybdate in the reagent exert a very marked effect on the formation of phospho- and silicomolybdic acids, and on the ease of reduction of these heteropoly acids and of the molybdate ion itself. In order to establish the most suitable composition for the single-solution reagent, experiments were carried out varying the acid and molybdate concentrations systematically in the presence of a fixed amount of ascorbic acid. Aliquots (10 ml.) of distilled water, phosphate solution (5 μg PO_4^{3-}-P/ml.) and silicate solution (120 μg SiO_3^{2-}-Si/ml.) were treated with mixed reagents containing varied volumes of 2 % ammonium molybdate and 4 N sulphuric acid, together with 5 ml. of 0·1 M ascorbic acid; the solutions were diluted to 50 ml. After 24 h their optical densities were measured at 827 mμ in a 1 cm cell. The results, which are given in Table 1, show that if low reagent blanks and freedom from interference by silicate are to be realized, the final concentrations of acid and molybdate in the solution should be approximately 0·40 N and 0·10 % respectively. Variation of the final ascorbic acid concentration in the range 0·004 to 0·03 M has no effect on the optical density attained. In all subsequent work 8 ml. of a reagent 2·5 N in sulphuric acid, 0·60 % in ammonium molybdate, and 0·03 M in ascorbic acid was used for a final volume of 50 ml.

TABLE 1. DETERMINATION OF OPTIMUM ACID AND MOLYBDATE CONCENTRATIONS FOR ESTIMATION OF PHOSPHATE USING ASCORBIC ACID

Optical densities measured at 827 mμ (1 cm cell).

ml. of 4 N sulphuric acid		ml. of 2 % ammonium molybdate						
		1	2	3	4	5	7	10
2·5	Blank	0·010	—	0·024	—	>2·5	—	—
2·5	50 μg PO_4^{3-}-P	0·445	—	0·475	—	>2·5	—	—
2·5	1·2 mg SiO_3^{2-}-Si	0·074	—	>2·5	—	>2·5	—	—
5·0	Blank	0·006	0·006	0·006	0·016	0·018	0·025	>2·5
5·0	50 μg PO_4^{3-}-P	0·246	0·452	0·456	0·455	0·455	0·454	>2·5
5·0	1·2 mg SiO_3^{2-}-Si	0·017	0·041	0·043	0·204	0·609	1·59	>2·5
7·5	Blank	0·004	—	0·009	—	0·011	0·015	>2·5
7·5	50 μg PO_4^{3-}-P	0·061	—	0·441	—	0·442	0·477	>2·5
7·5	1·2 mg SiO_3^{2-}-Si	0·013	—	0·024	—	0·139	0·355	>2·5
10·0	Blank	0·005	—	0·007	—	0·012	0·008	0·005
10·0	50 μg PO_4^{3-}-P	0·005	—	0·301	—	0·450	0·438	0·440
10·0	1·2 mg SiO_3^{2-}-Si	0·010	—	0·012	—	0·017	0·018	0·022
15·0	Blank	0·003	—	0·005	—	0·011	0·015	0·017
15·0	50 μg PO_4^{3-}-P	0·006	—	0·038	—	0·170	0·392	0·447
15·0	1·2 mg SiO_3^{2-}-Si	0·006	—	0·011	—	0·015	0·019	0·028

Using this reagent, colour development is complete in both sea water and distilled water after 24 h at 20° C, or after 30 min at 60° C. The colour once formed is stable for at least 60 h. The calibration curve is independent of changes in batches of reagents.

METHODS

All measurements of optical density were made with a Unicam S.P. 500 spectrophotometer modified to use 7·62 and 15·24 cm cells. Distilled water was used in the compensating cell.

Reagents

Sulphuric acid 5 N. Dilute 70 ml. of concentrated sulphuric acid to 500 ml.

Ammonium molybdate (4%). Dissolve 20 g of ammonium molybdate A.R. in distilled water and dilute to 500 ml. Store the solution in a Pyrex glass reagent bottle.

Ascorbic acid 0·1 M. Prepare a solution of 1·76 g of ascorbic acid in 100 ml. of distilled water. It is preferable to prepare this solution on the day it is needed, but if this is not possible, the solution may be preserved for about 10 days at 0° C.

Mixed reagent. Mix thoroughly 125 ml. of 5 N sulphuric acid and 37·5 ml. of 4% ammonium molybdate solution. Add 75 ml. of ascorbic acid solution and dilute to 250 ml. The mixed reagent does not keep well and should be prepared within an hour of using.

Standard solutions

Stock phosphate solution. Dissolve 0·1757 g of potassium dihydrogen phosphate in distilled water and dilute to 1 l. This solution, which contains 40 mg PO_4^{3-}-P/l., should be preserved in a dark glass bottle.

Dilute phosphate solution. Prepare, by dilution of the stock phosphate solution, a solution containing 0·2 mg PO_4^{3-}-P/l. This standard solution should be prepared daily.

Treatment of apparatus

Allow apparatus, which is to be used in the determination, to stand overnight filled with concentrated sulphuric acid, and then rinse thoroughly. It is preferable to keep a set of graduated flasks to be used only for the determination of phosphate; after use, they should be washed well and kept filled with distilled water until required again. If this is done, the treatment with sulphuric acid is only required occasionally.

Determination of phosphate

Pipette 40 ml. of the sea-water sample into a 50 ml. graduated flask, add 8 ml. of the mixed reagent from a tilt measure and dilute to volume. Allow the colour to develop for 24 h at room temperature (20° C). Measure the optical density of the solution at 827 mμ. Determine the reagent blank in the same manner using freshly distilled water. Calibrate the method using 5, 10 and 20 ml. aliquots of the dilute standard phosphate (1, 2 and 4 μg. PO_4^{3-}-P respectively). To correct the calibration curve for use with sea water multiply the observed optical densities by the appropriate salt error factor (Table 3). The calibration curve remains constant and is independent of changes in the batches of reagents. The colour development may be carried out more rapidly at 60° C if desired. Transfer the solution to a loosely stoppered Pyrex flask (treated as described above) and heat it in a water bath at 60° C for 30 min. After cooling, measure the optical density of the solution at 827 mμ.

Beer's law and reproducibility

Calibration runs were carried out using 1–25 μg PO_4^{3-}-P in 40 ml. of distilled water. The colour was developed for 24 h at room temperature and the optical densities were then measured at 827 mμ in cells of appropriate length. The results, which are given in Table 2, show a satisfactory reproducibility and show that Beer's law is obeyed up to a concentration of at least 0·5 p.p.m. PO_4^{3-}-P. Similar figures were obtained when the colour was developed at 60° or 90° C.

TABLE 2. DETERMINATION OF PHOSPHORUS

PO_4^{3-}-P μg	No. of determinations	Mean optical density* (7·62 cm cell)	Mean deviation (%)	Deviation from linearity (%)
1	4	0·134	0·6	+0·1
2	4	0·267	0·5	−0·3
3	4	0·403	0·3	+0·3
4	4	0·534	0·5	−0·3
5	3	0·671†	0·5	+0·2
10	3	1·342†	0·2	+0·2
15	3	2·019†	0·3	+0·5
20	3	2·667†	0·3	−0·4
25	3	3·330†	0·3	−0·5

* Less reagent blank.

† Measured in 1 cm cell and calculated for 7·62 cm cell.

TABLE 3. SALT ERROR CORRECTIONS

Sea water, Cl‰	0	5	10	15	19·3
Mean optical density for 25 μg, PO_4^{3} -P*	0·437	0·424	0·420	0·417	0·419
Salt error, %	0	−2·9	−3·9	−4·6	−4·1
Correction factor	1·00	0·970	0·961	0·954	0·959

* 1 cm cell.

SALT ERROR

The effect of the salts of sea water on the intensity of the molybdenum-blue colour, developed by the mixed reagent, has been studied. Filtered sea water, low in phosphate, was diluted with distilled water to give sea waters having chlorinities of 5, 10, 15 and 19·3‰. Duplicate phosphate determinations were made on 40 ml. portions of these waters and on 40 ml. aliquots which had been enriched with 25 μg PO_4^{3-}-P. Colour development was carried out at room temperature. The results, which are summarized in Table 3, indicate that, as with the metol method, the salt error is not a linear function of chlorinity. For oceanic waters it is small compared with the salt error of the stannous chloride procedure (*ca.* 20%).

INTERFERENCES

The interference of several ions, known to interfere in molybdenum-blue methods for the determination of phosphorus, has been investigated using the ascorbic acid reagent. Experiments were performed using 40 ml. aliquots of distilled water containing these ions, both alone, and in the presence of 5 μg of PO_4^{3-}-P. The results (Table 4) indicate that of the elements capable of forming molybdenum-blue complexes, only arsenic interferes appreciably.

In every case the interference is much less than that found with either the stannous chloride or metol methods. The effects of these ions at their normal concentrations in sea water will be quite negligible. High concentrations of copper (500 p.p.m.) seriously reduce the intensity of colour formed, presumably by destruction of the ascorbic acid. Even as much as 500 p.p.m. of ferric iron has no effect. Since the colour development is rather prolonged and takes place in 0·24 N acid, it was thought that hydrolysis of the organic phosphorus compounds present in sea water might occur. This did not prove to be the case, however, since determinations carried out in distilled water, to which fairly readily hydrolysable organic phosphorus compounds had been added, gave no molybdenum-blue colour even after heating for several hours at 70° C.

TABLE 4. INTERFERENCES OF CERTAIN ELEMENTS

			Optical density at 827 mμ (7·62 cm cell)		
Element	Added as	Concentration of element (p.p.m.)	No added phosphate	5 μg PO_4^{3-}-P	Difference
—	—	—	0·055	0·710	0·655
Copper	$CuSO_4$	500	0·057	0·600	0·543
Iron	$FeCl_3$	50	0·056	0·715	0·659
Arsenic	Arsenate	1	0·107	0·814	0·707
Arsenic	Arsenite	0·1	0·059	0·715	0·656
Silicon	Silicate	10·0	0·057	0·710	0·653
Vanadium	Vanadate	500	0·085	0·743	0·658
Germanium	Germanate	1·0	0·057	0·715	0·658

SUMMARY

It has been found that a reagent containing sulphuric acid, ammonium molybdate and ascorbic acid may be used as a single-solution reagent for the determination of phosphate in sea water. Development of the molybdenum-blue colour is complete in 24 h at room temperature and in 30 min at 60° C; the colour is stable for at least 3 days. Beer's law is obeyed closely up to at least 500 μg PO_4^{3-}-P/l. The salt error is approximately 4% with sea water of chlorinity 19·3‰. The interference due to either arsenate or silicate at their concentrations in sea water is negligible.

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THE FUNCTION OF THE HEART-BODY IN POLYCHAETES

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(Text-figs. 1–9)

Loose, spongy intravasal tissues are common in polychaetes. In some this tissue is localized and forms a discrete plug or strand known as the heart-body. Such structures occur in the anterior part of the dorsal vessel in terebellids, ampharetids, cirratulids, amphictenids and flabelligerids.

Many authors have described the heart-body in different species and speculated about its function. Picton (1898) examined in some detail the heart-body in the cirratulid *Audouinia tentaculata* and in the terebellids *Polymnia nebulosa* and *Terebella meckelii*. He concluded that in *Polymnia* at least, the heart-body is formed by infolding from the epithelium on the outside of the vessel, so that it is comparable with the chloragogen cells which, in many polychaetes, clothe the outside of the vessels. Salensky (1884) working on *Terebella*, came to the same conclusion. Eisig (1887) also pointed out that such extravasal chloragogen cells are absent in species with intravasal heart-bodies, and compares the chloragosomes or yellow-green granules found in the chloragogen cells with the granules found in the heart-body. Romieu (1923) comes to similar conclusions. Fauvel (1897) concluded from his study of the ampharetids, that the heart-body served a valvular function, and suggested that it also removed and stored substances extracted from the blood, but was not directly related to the amoebocytes. Schneider (1897, 1899), on the other hand, concluded that the organ was related to the phagocytic and excretory systems in the amphictenid, *Pectinaria*. Picton (1898) concluded that the greenish granules were removed from the heart-body to the coelomic fluid, and from there to the outside by the nephridia, since the quantity of pigment did not appear to be greater in older worms.

In addition to the greenish or yellow granules in the heart-body, Picton (1898) also found fat, iron, and brownish 'chitinous bodies'. Iron was also detected by Bloch-Raphael (1939) and Romieu (1923), and its occurrence suggests an haematopoietic function. Schneider (1897), who also found iron in the heart-body of *Pectinaria*, claimed that this increased after injection of iron saccharate into the coelom; Meyer (1887) also suggested, from his study of the cirratulid *Chaetozone*, that the heart-body is an haematopoietic organ.

The histological structure of the heart-body does not differ greatly in polychaetes belonging to different families, although it is variable in size and extent within the dorsal vessel, and may also increase with age (Ashworth, 1904). It is perhaps most extensive in the cirratulids, but seems best developed in the terebellids. The appearance of the heart-body of *Audouinia tentaculata* in transverse section is shown in Fig. 1. The organ in cirratulids extends throughout the region in which the dorsal vessel supplies vessels to the branchiae, and the general occurrence of heart-bodies in those polychaetes with specialized gill structures suggests that the organ has a mechanical function, as Picton (1898) concluded; certainly at systole the lumen of the dorsal vessel is completely obliterated by the wall of the vessel contracting on to the heart-body. It is perhaps not unreasonable to suppose that such loosely compacted tissue may have acquired other functions; a physiological parallel may be found in the vertebrate bone-marrow.

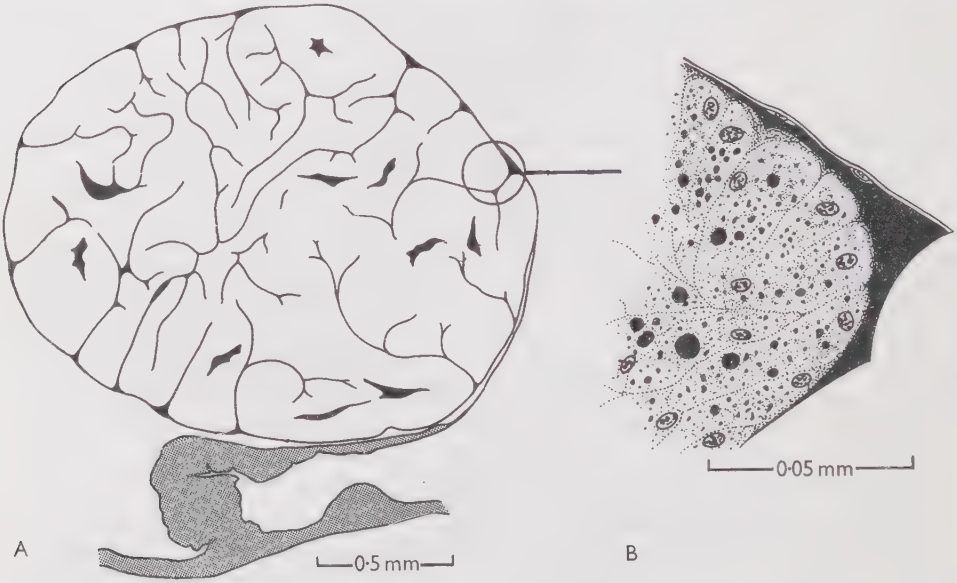

Fig. 1. *Audouinia tentaculata*, heart-body; A, section of whole organ, with gut-wall shaded; B, enlarged detail of part of A.

In the present work, pigment analyses have been made of the heart-bodies from the following species representing the main families of polychaetes in which discrete heart-bodies are found: *Amphitrite johnstoni* Malmgren, *Terebella lapidaria* L., *Lanice conchilega* (Pallas) (Terebellidae); *Audouinia tentaculata* (Montagu), *Cirratulus cirratus* (O. F. Müller) (Cirratulidae);

Melinna palmata (Grube) (Ampharetidae); and *Flabelligera affinis* Sars (Flabelligeridae). Unfortunately, insufficient specimens of *Pectinaria* were collected to make pigment examination of the heart-body possible. The heart-bodies of *Amphitrite johnstoni* and *Audouinia tentaculata* have also been examined histologically and histochemically. Similar chemical methods have been applied to other tissues in all these worms for comparison, and the work extended to *Polycirrus caliendrum* Clap. (Terebellidae), *Myxicola infundibulum* (Renier) (Sabellidae) and *Arenicola marina* L., none of which has a discrete heart-body, although there is a little intravasal tissue in the latter genera; *Polycirrus* has no vascular system. The extravasal tissue present in old *Arenicola* has also been examined histologically (Fig. 2), histochemically, and subjected to the same procedures to be described for *Amphitrite*.

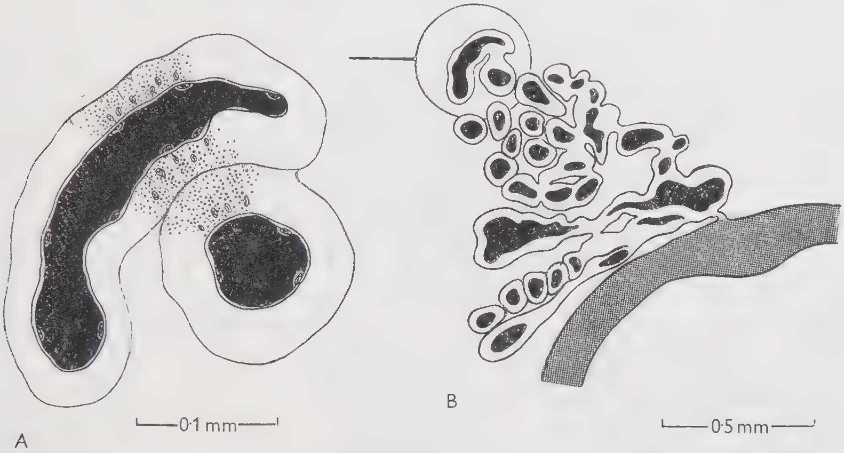

Fig. 2. *Arenicola marina*, extravasal tissue; A, detail of part of tissue; B, section of tissue, with gut wall shown shaded.

The work described in this paper was done at the Plymouth Laboratory, and we would like to express our appreciation of the facilities and help afforded to us by the Director and Staff, in particular Mr F. A. J. Armstrong for copper analysis; to Miss M. L. Weir for preparing the sections represented in Fig. 2, and to Mr A. C. Briggs for collecting material. We are also grateful to Mr W. H. Lockwood, of the Department of Chemical Pathology, University College Hospital Medical School, London, for some helpful discussion.

METHODS

The tissues from the animals examined were extracted by the methods described by Kennedy & Vevers (1953, 1954). Whenever possible, the pigments detected were isolated, melting points determined, and some characteristic derivatives prepared. Absorption spectra were determined in the Beck–Hartridge Reversion Spectroscope, in the Unicam S.P. 500 Spectrophotometer, and in the Beck microspectroscope.

RESULTS

The main results are presented in Table 1.

Amphitrite johnstoni

Heart-body

Ether-acetic acid extracts from fifteen heart-bodies were washed with water containing some potassium acetate. The hypophase was bright yellow and showed a fine green fluorescence in u.v. light. The epiphase was red-fluorescent in u.v. light, and gave a spectrum (Hartridge):

I	II	III	IV
628·3	576·5	536	504 mμ

Spotted on the long-paper chromatograph (Kennedy, 1953*a*) with markers of coproporphyrins I and III and mesoporphyrin IX in adjacent and mixed spots, and run in a solvent phase of 2:6-lutidine/water at 26° C, the extract gave three spots (Fig. 3). Two spots corresponded with the two coproporphyrins, and another spot was detected between the coproporphyrin III and mesoporphyrin IX spots, where one would expect to find porphyrins with three carboxyl groups.

The crude ether extract was spotted on Whatman No. 4 paper, with markers of coproporphyrin I, III, chlorin-e_6 and haematoporphyrin IX, and chromatograms run according to the method of Eriksen (1953). The crude extracts gave four spots: two corresponded with those given by coproporphyrins I and III, there was one spot at the tricarboxylic level (see below, Band 3), and one dark-coloured non-fluorescent spot joining the latter with that given by coproporphyrin III (Fig. 4). This dark spot is probably a haematin.

Extracts of a further fifteen heart-bodies in methanol-sulphuric acid were diluted with an equal volume of water and extracted with chloroform. The chloroform extract was washed with 2% sodium chloride, and then with water, and concentrated *in vacuo*. This solution gave a spectrum (Hartridge):

I	II	III	IV
626·3	573·5	540	506·2 mμ

After a further washing, the esterified pigment was spotted on Whatman No. 1 C.R.L. slotted paper, and chromatograms run according to the method of

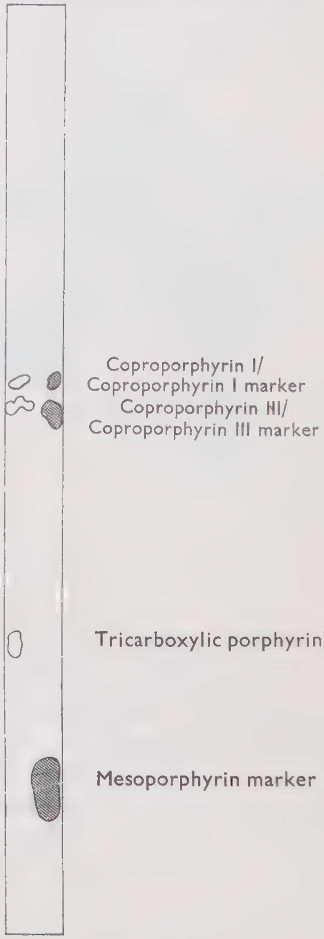

Fig. 3. Long-paper descending chromatogram of extract of *Amphitrite* heart-body, with adjacent markers.

Chu, Green & Chu (1951) for separating isomers of the coproporphyrins. Coproporphyrins I and III esters, and mesoporphyrin IX ester were used as markers. By this method, both the isomers I and III of coproporphyrin were found in the extract (Fig. 5). The chloroform solution of the pigment esters was then chromatographed on a column of magnesium oxide grade III (Nicholas, 1951) packed in chloroform. The chromatogram was developed with methanol-in-chloroform, 0·5, 1 and 2%, successively, and the fractions collected separately as the bands were eluted. Four fractions were obtained as follows:

Band 1. Brownish red fraction passing quickly down the column and out. Red-fluorescent in u.v. light. Hartridge spectrum:

I	II	III	IV
645	586	545	504 mμ (Chlorin-type)

TABLE 1. LOCALIZATION OF THE PIGMENTS IN THE TISSUES

	Uro I	Copro I	Copro III	3-COOH	Proto IX	Haematin	Yellow green-fluorescent	Blue-fluorescent
Amphitrite								
Heart-body	–	+	+	+	–	+	+	–
Coelomic cells (pink)	–	–	+	–	–	+	–	–
Coelomic cells (brown)	–	–	+	–	–	+	–	–
Blood	–	–	–	–	–	+ (Hb)	–	–
Body wall	–	–	+	–	–	+	+	–
Gut	–	–	–	–	–	–	–	–
Terebella								
Heart-body	–	–	+	–	–	–	–	–
Coelomic cells	–	–	–	–	–	+	–	–
Blood	–	–	–	–	–	+ (Hb)	–	–
Body wall	–	–	–	–	–	–	–	–
Gut	–	–	–	–	–	–	–	–
Lanice								
Heart-body	–	–	+	–	–	–	–	–
Gut	–	–	+	–	–	–	–	–
Cirratulus								
Heart-body	–	–	+	–	–	+	–	–
Body wall + gut	–	–	–	–	–	+	–	–
Audouinia								
Heart-body	–	+	+	–	–	–	–	+
Blood	–	+	+	–	–	+ (Hb)	–	–
Body wall	–	–	–	–	–	?	–	–
Gut	–	+	+	–	–	–	–	+
Melinna								
Heart-body	–	–	+	–	–	–	+	–
Body wall	–	–	–	–	–	–	–	–
Gut	–	–	–	–	–	–	–	–
Flabelligera								
Heart-body	–	–	+	–	–	–	–	+
Body wall	–	–	–	–	–	–	–	+
Gut	–	–	+	–	–	–	–	–
Polycirrus								
Whole body	–	–	+	–	–	–	–	–
Body wall	–	–	+	–	–	–	–	–
Gut	–	–	+	–	–	–	–	–
Myxicola								
Anterior nephridia	–	–	–	–	–	–	–	–
Body wall + gut	–	–	+	–	–	+	–	–
Arenicola								
Extravasal tissue	–	+	+	+	–	+	–	–
Body wall (dark)	–	+	+	+	–	+	+	–
Body wall (pink)	–	+	+	+	–	+	+	–

(Hb) = from haemoglobin.

In the spectrophotometer, only the band at 643 mμ was detected, probably owing to impurity. This spectrum resembles that given by dioxymesoporphyrin:

I	II	III	IV
640	584	542	504 mμ (chlorin type)

This pigment could be an artefact arising during manipulation of the pigments.

Band 2. A wide pink band, obviously the main pigment present. This was collected after elution with 0·5 % methanol in chloroform. This gave a spectrum in the Unicam:

I	II	III	IV	Soret
622	569	535	501	405 mμ (aetio-type)

This is the spectrum given by coproporphyrin.

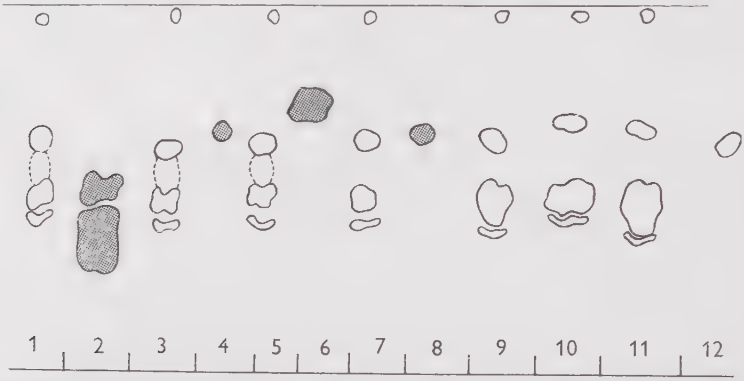

Fig. 4. Ascending paper chromatogram of *Amphitrite* heart-body and *Arenicola* tissue extracts. Solvent 2:6-lutidine/water at 26° C in atmosphere of NH_3. The small spots at the top are carotenoids; the other spots are as follows: (1) *Amphitrite* heart-body; (2) coproporphyrin III (lower) and coproporphyrin I; (3) *Amphitrite* heart-body ; (4) chlorin-e_6; (5) *Amphitrite* heart-body; (6) haematoporphyrin; (7) *Amphitrite* heart-body; (8) chlorin-e_6; (9) *Arenicola* extravasal tissue; (10) *Arenicola* body wall (light); (11) *Arenicola* body wall (dark); (12) *Amphitrite* heart-body 3-COOH spot.

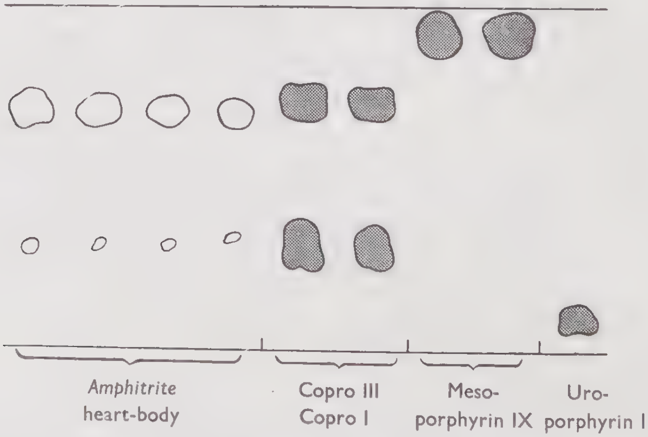

Fig. 5. Separation of porphyrin isomers by Chu, Green & Chu technique (1951).

Band 3. A third red-fluorescent band appeared which travelled much more slowly than the preceding band, and was eluted with 2 % methanol in chloroform. This band gave a spectrum in the Unicam:

I	II	III	IV	Soret
622	572	538	503	404 mμ (aetio-type)

Band 4. Some pigment remained close to the top of the column and could not be eluted even with 5 % methanol in chloroform. The column was pushed out and this band cut off with a razor blade. The brown material at the very top of the column was discarded, and the red-fluorescent material eluted with chloroform containing a trace of formic acid. The chloroform extract was washed with water containing a little potassium acetate and then with water to remove the formic acid. The remaining chloroform solution gave a spectrum in the Unicam:

I	II	III	IV	Soret
621	568	535	500	402 mμ (aetio-type)

This spectrum resembles that of coproporphyrin, and it is possible that this is unesterified pigment in view of its chromatographic behaviour, although free coproporphyrin is normally somewhat insoluble in chloroform.

Band 1 containing the pigment which resembles dioxymesoporphyrin could be, as already mentioned, an artefact due to oxidation during the extraction process. In the first paper chromatograms carried out, no spot at the 2-carboxyl level was obtained from the crude *Amphitrite* heart-body extracts. However, in later chromatograms a trace of red fluorescence at the 2-carboxyl level was in fact obtained. This would lend credence to the view that this pigment is indeed an oxidation artefact.

Band 2, which contains the bulk of the porphyrin present in the heart-body, was shown, by the technique of Chu *et al.* (1951), to be a mixture of coproporphyrins I and III, with the III isomer predominating (Fig. 5). The isomers were separated by the Chu *et al.* (1951) method, the spots of the isomers were cut out with scissors, and those of each isomer pooled. The porphyrin esters were eluted with chloroform-methanol mixture 100:2, and the methanol washed out with water containing some potassium acetate to prevent the coproporphyrin from passing into the water phase. After a further washing with water, the chloroform solutions were roughly dried by filtering through chloroform-soaked filter papers, and the pigments crystallized. Coproporphyrin III was crystallized by evaporating to a volume of 3 ml. and adding an equal volume of dry ether, stirring all the time. The mixture was placed in the ice chest for some hours, when clusters of coproporphyrin III tetramethyl ester were obtained (Rimington, 1939). The melting point after one recrystallization was 136° C. Vannotti (1954) gives the melting point of coproporphyrin III tetramethyl ester as 137° C. There was too little of the coproporphyrin I isomer for crystallization.

The pigment ester from Band 3 was hydrolysed by dissolving it in concentrated HCl and allowing it to stand at room temperature overnight. The HCl was removed in a vacuum desiccator over solid KOH, and the resultant pigment dissolved in a little 2:6-lutidine. The solution was spotted on to Whatman No. 4 paper with markers of chlorin-e_6, coproporphyrin I, coproporphyrin III, and haematoporphyrin, and the chromatograms were run at 26° C in 2:6-lutidine/water in an atmosphere of ammonia (Fig. 4). The pigment gave only one spot which travelled further than those of the tetracarboxylic coproporphyrin III, and took up a position approximating to that given by chlorin-e_6 which has three carboxyl groups. It will be noted that spots of the crude extract from *Amphitrite* heart-body also gave this spot, but in this case it did not travel quite as far as the tricarboxylic chlorin spot. This is most likely due to retardation by the other pigments travelling behind it. From these data it is permissible to conclude that the pigment from Band 3 is a tricarboxylic porphyrin. The free porphyrin has an HCl number of 0·5–1·0.

Band 4 gave a very indeterminate spot on an ascending lutidine chromatogram, about the 4-carboxyl level, the fluorescence of which was not particularly intense. It would, therefore, seem that this is in fact some unesterified coproporphyrin.

The pigments obtained from the heart-body tissue were, therefore, coproporphyrins I and III, coproporphyrin III being the main pigment present, and a tricarboxylic porphyrin.

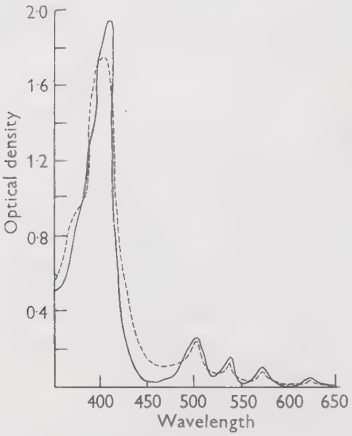

Fig. 6

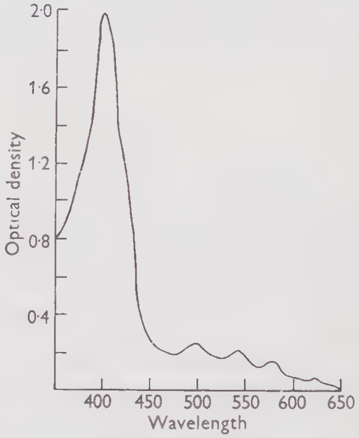

Fig. 7

Fig. 6. Absorption curves of main heart-body pigment ester (Band 2) in $CHCl_3$ (broken line) and of coproporphyrin I tetramethyl ester in $CHCl_3$ (continuous line).

Fig. 7. Absorption curve of tricarboxylic porphyrin ester (Band 3) in $CHCl_3$.

Remaining tissues

The coelomic cells, pink and brown (see Dales, 1957); the blood, the body wall, and the gut were all examined by the methods which have already been described for the heart-body. These results are summarized in Table 1, from which it will be seen that with the exception of the gut all these tissues contain haematin, shown to be protohaematin by histochemical tests. This haematin, treated with hydrazine hydrate gave a spectrum, Hartridge:

I	II
555	530 mμ

The haematin from the blood was certainly formed from the haemoglobin in the course of extraction.

Coproporphyrin III was present in the coelomic cells and the body wall in relatively small amounts as compared with that in the heart-body, and was entirely absent from the blood. The tricarboxylic pigment did not occur in any of these tissues.

The body wall and the heart-body contained a bright yellow intensely green-fluorescent pigment resembling in behaviour the pigment found in *Arenicola* and in holothurians. It is intended to investigate this pigment later.

Terebella lapidaria

Coproporphyrin III was the only porphyrin present, occurring only in the heart-body. Protohaematin was detected in the coelomic cells, and in extracts of the blood, in which case it was formed from haemoglobin.

Lanice conchilega

Coproporphyrin III was the only porphyrin detected, and occurred in the heart-body and the gut.

Cirratulus cirratus

Coproporphyrin III was detected in the heart-body together with protohaematin. Protohaematin was also detected in the body wall plus gut.

Audouinia tentaculata

The heart-body, the blood and the gut contained coproporphyrins I and III, with the III isomer predominating. Protohaematin was derived from the haemoglobin, in the blood extracts, and this pigment possibly occurred in the body wall. The heart-body and the gut also contained a colourless compound giving an intense blue fluorescence in u.v. light (c.f. *Flabelligera*).

Melinna palmata

Coproporphyrin III was detected in the heart-body, but not in the body wall or the gut. The heart-body in addition contained some of the yellow, green-fluorescent pigment found in *Amphitrite*.

Flabelligera affinis

The heart-body and the gut contained coproporphyrin III. Ether-acetic extracts of the heart-body and of the body wall when washed with water showed an intense bright blue fluorescence in u.v. light apparently masking or quenching that due to coproporphyrin. The residue was a dark green in colour, and was non-fluorescent. Chromatographed in the long paper apparatus in 2:6-lutidine/water at 23° C in an atmosphere of ammonia, an intensely blue-fluorescent spot was formed at R_F 0·6, which is at the 4-carboxyl level. Again, the fluorescence of the coproporphyrin seemed to be masked. On washing the ether-acetic acid extract with water, the blue-fluorescent pigment became hypophasic and could thereby conveniently be separated. The ether epiphase when concentrated *in vacuo* gave the spectrum (Hartridge) of coproporphyrin, and when examined chromatographically, only the III isomer could be detected. The water hypophase containing the blue-fluorescent pigment was extracted with *n*-butanol, when all the pigment went into the butanol phase. Evaporated to dryness *in vacuo* and redissolved in butanol, no definite absorption spectrum was given in the spectrophotometer (Fig. 8). This pigment is interesting in that it is extremely soluble in both fat solvents and in water, and it has a definite R_F value. Fox, Crane & McConnaughey (1948) described non-carotenoid blue-fluorescent chromolipoids from *Thoracophelia mucronata*. These pigments have single sharp maxima in the u.v. at from 310 to 300 mμ or below, but the pigment of *Flabelligera* does not seem to be of this type since it has no definite maxima, and is extremely soluble in water. It is possible that the pigment is related to fluorocyanine. Phaeophorbide-*a* was also detected in the ether epiphase, and the dark green non-fluorescent residue from the ether extraction when treated with mineral acids gave the absorption spectrum of phaeophorbide-*a*. Copper was detected in the ash. The dark green non-fluorescent pigment in the residue must, therefore, be a copper phaeophorbide-*a*. This pigment has already been detected in the mollusc *Akera bullata* (Kennedy & Vevers, 1956).

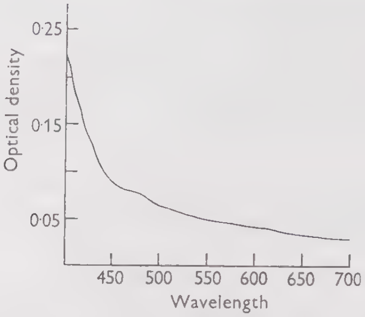

Fig. 8. Absorption curve of blue-fluorescent pigment from *Flabelligera* in *n*-butanol.

Polycirrus caliendrum

Coproporphyrin III was the only porphyrin detected, and occurred in extracts of the whole body, the body wall, and the gut. (*Polycirrus* is without vascular system, and hence heart-body.)

Myxicola infundibulum

Coproporphyrin III and chlorohaematin were found in the body wall plus gut, but not in the anterior nephridia. It is most likely that the haematin came from the body-wall tissue. Coproporphyrin III has been found previously in the gut (Kennedy & Vevers, 1954), and chlorohaematin can be prepared from the blood (Kennedy, 1953*b*).

Arenicola marina

Ashworth (1904) reports intravasal tissue increasing with age within the hearts of *Arenicola*. This was found in insufficient quantity for extraction. Extravascular tissue, also increasing with age, related to certain vessels from the hinder part of the intestine, occurs in larger amounts, and extracts were made of this tissue and of the body wall of pink and dark worms (Fig. 2).

Extravasal tissue was removed from a number of worms, homogenized, washed with sea water, and centrifuged. This process was repeated until the tissue was freed from blood. No absorption band was visible with the microspectroscope; the spectrum of oxyhaemoglobin was absent. After the addition of a drop of hydrazine hydrate, the pink colour of a haemochromogen appeared. This gave a two-banded spectrum in the microspectroscope at

I	II	
555	525 $m\mu$	(order of intensity I–II)

This is the spectrum given by protohaemochromogen, indicating that protohaem is present in the tissue, most probably as haematin. A drop of 2 N-HCl, added to a smear of the extravasal tissue on a slide, produced the spectrum of acid haematin with a strong maximum of 660 $m\mu$. This is characteristic of acid protohaematin. The smear of the extravasal tissue allowed to dry on a slide shows very little, if any, fluorescence in u.v. light. A drop of ethanol, when added, caused the appearance almost immediately of a bright yellowish green fluorescence resembling that already described in *Amphitrite* and *Melinna*. This fluorescent pigment was washed off with ether, after which no fluorescence was apparent. A drop of formic acid produced a bright red fluorescence at once. Examined with the microspectroscope the absorption spectrum of coproporphyrin became apparent, with a slight shift of the bands towards the red due to formic acid. The non-appearance of coproporphyrin spectrum on examination of the fresh tissue may be due to the fact that the porphyrin is almost certainly present in combination with protein in the extravasal tissue. The addition of formic acid would not be sufficient to break down a haematin in the cold.

Fresh *Arenicola* blood gives a spectrum of oxyhaemoglobin:

I	II	
578	540 mμ	(Hartridge)

The addition of a drop of hydrazine hydrate produced the haemochromogen:

I	II	
555	525 mμ	(Hartridge)

and bubbling carbon monoxide through a sample of haemolysed blood produced a spectrum:

I	II	
570	540 mμ	(Hartridge)

of carboxyhaemoglobin. These experiments were sufficient to indicate that the blood contains a haemoglobin with protohaem as its prosthetic group. A drop of fresh blood treated with dilute HCl produced a brown coagulum. This did not fluoresce with u.v. light, even after the addition of formic acid, and this is conclusive proof that the blood does not contain a free porphyrin.

These experiments indicate that the porphyrin in the extravasal tissue does in fact come from that tissue and not from the blood. The tissue also contains protohaematin.

Coproporphyrin I and coproporphyrin III, with the III isomer predominating, were found in the extravasal tissue and the body wall of light and dark animals. All these tissues also contained the tricarboxylic porphyrin already described from the heart-body of *Amphitrite*, and in addition they contained protohaematin. The body wall of both dark and light animals also produced the yellow, green-fluorescent pigment described in *Amphitrite* and *Melinna*. Such a pigment has been described by Lignac (1945) and van Duijn, Havinga & Lignac (1951) in *Arenicola* and called arenicochrome. It may well be that it is this pigment which occurs in *Amphitrite* and *Melinna*. This will be the subject of a future investigation.

Ether-acetic acid extracts of the extravasal tissue and the body wall of dark and light animals were washed with water, concentrated and spotted on Whatman No. 4 filter paper with markers of coproporphyrin I and III, chlorin-e_6, and haematoporphyrin, and chromatograms were run using the ascending method of Eriksen (1953) (Fig. 4). In each case spots of coproporphyrin I and coproporphyrin III were obtained, coproporphyrin III predominating, together with a spot at the tricarboxylic level. The ether extracts were evaporated to dryness and the pigment esterified by adding a mixture of methanol 19 parts: concentrated sulphuric acid 1 part, and allowing the solution to stand at room temperature for 48 h. The esterified pigments were extracted by diluting with an equal volume of water and shaking with chloroform. After washing the chloroform extract with 2% sodium chloride followed by water, and drying the solution roughly by passing it through chloroform-soaked paper, the pigments were chromatographed on a column of MgO, grade III (Nicholas, 1951). Apart from a dark brown band at the top of the column which did not move when the chromatogram was developed with the chloroform-methanol mixture as before, only one broad, pink, red-fluorescent band developed. When collected in chloroform this band gave an absorption spectrum (Hartridge):

I	II	III	IV
622·3	565·5	533	501 mμ

From the paper-chromatographic data and this spectrum, the pigment can be identified as coproporphyrin.

From the body wall of the dark animals a large amount of coproporphyrin isomers was isolated; much less, although still an appreciable quantity, could be extracted from the light (pink) animals. When crystallized as described for *Amphitrite*, the coproporphyrin III tetramethyl ester gave a melting point of 137·9° C. The coproporphyrin I again was present in too small a quantity to be isolated.

From this work it may be seen that coproporphyrin III is the predominating pigment in the heart-bodies of all the species examined. The tricarboxylic porphyrin was only detected in *Amphitrite* and *Arenicola* extravasal tissue, but this could be because much larger amounts of these worms were available, and only a few specimens of some of the other species could be obtained.

DISCUSSION

The chief interest in this work is centred in the presence of relatively large amounts of coproporphyrin III, together with traces of coproporphyrin I, a tricarboxylic porphyrin, and haematin in the heart-body. The fact that all these pigments can be isolated from the heart-body tissue is very strong evidence in support of the view expressed by Meyer (1887) that the heart-body is an haematopoietic organ. A possible scheme for the biosynthesis of haem is that of Dresel (1955) represented in Fig. 9. In this scheme porphobilinogen (PBG) is converted into an 8-carboxyl leuco-compound or prouroporphyrin III, which is then progressively decarboxylated each time to the III leuco-compound and finally dehydrogenated, with the incorporation of Fe leading to haem. Dresel (1955) considers that observations of porphyrins with from 8- to 2-carboxyl groups in many different biological situations lend support to such a scheme. If this system proceeds normally, only minute traces of free porphyrins are formed. However, in the porphyrias, where

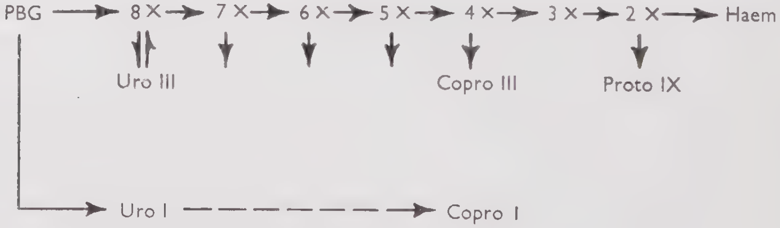

Fig. 9. A scheme for the biosynthesis of haem (Dresel, 1955).

aberrations of the biosynthetic process occur, free porphyrins are formed, that is to say, the leuco-compounds are oxidized to the coloured free porphyrins which then find their way into the tissues. Once these free porphyrins have been formed they apparently cannot play any further part in the synthesis of the haem molecule. Eriksen (1955) put forward some evidence for the conversion of free uroporphyrin and coproporphyrin to protoporphyrin in the biosynthetic cycle, but this has not been supported, and has been criticized by Schwartz & Ikeda (1955), by Shemin (1955) and by Dresel (1955). The metabolism of the series I porphyrins appears to follow a different pathway in which the free porphyrins, at any rate uroporphyrin I and coproporphyrin I, appear. Lockwood & Rimington (1957) have succeeded in purifying an enzyme which converts PBG to uroporphyrin, and for which they propose the name porphobilinogenase (PBGase). This enzyme produces uroporphyrin III from PBG apparently without trace of uroporphyrin I. If, however, the enzyme is heated for 15 min at 55° C, it produces from PBG at 37° C, uroporphyrin I, with a trace of uroporphyrin III, and no ether-soluble porphyrin is formed by either the heated or unheated enzyme. PBGase was prepared from chicken erythrocytes, and Lockwood (private communication) has shown that with Conger-eel erythrocytes, addition of PBG after haemolysis and incubation at room temperature produces large amounts of coproporphyrin III and protoporphyrin IX, with practically no uroporphyrin.

In the porphyrias, where the free porphyrins are formed, these may be both series I and series III according to the type of syndrome. In congenital porphyria, which is a gene-controlled disturbance of the haematopoietic system, quantities of uroporphyrin I and coproporphyrin I are formed, and Rimington & Booij (1957) found that a haemolysate prepared from the blood of a case of congenital porphyria produced considerable quantities of these porphyrins together with the series III isomers. Photosensitization occurs as well in congenital porphyria, and PBG is absent. In idiopathic porphyria, series III isomers predominate, and PBG is present. These findings give further support to Dresel's scheme (Fig. 9), and for the suggestion that series I isomers have a separate metabolic route. Booij & Rimington (1957) suggest that because a haemolysate of congenital porphyria blood incubated with PBG at 37° C yields a mixture of series I and III porphyrins, 'the defect is present in some at least of the circulating cells and is not confined to processes occurring in the bone marrow during early maturation of the erythrocytes'. All these studies have an important bearing on the present problem of the function of the polychaete heart-body, and on the occurrence of porphyrins in marine invertebrates. It is possible that in the polychaetes with haemoglobin which we have examined, there exists a kind of idiopathic porphyria in which the haem biosynthesis system is inefficient. In such a system the intermediates in the chain of reactions in Fig. 9, namely, coproporphyrin III, coproporphyrin I, and the tricarboxylic porphyrin, have been deposited in the tissues in their oxidized form and their metabolism to haem cannot, consequently, be carried further. Kench, Langley & Wilkinson (1953) found that extracts of stools of a porphyria patient, when chromatographed on paper, gave the whole gamut of porphyrins from 8-carboxyl down to 2-carboxyl. In molluscs, if free porphyrin is present it is uroporphyrin I which occurs (Kennedy & Vevers, 1954; Kennedy, 1958). In molluscs which have a shell the porphyrin is laid down in the shell, very often in a definite pattern, and in those molluscs which are without shells or have very reduced shells, such as the tectibranchs and land slugs, if uroporphyrin I is present it occurs in the integument. The deposition of uroporphyrin in these molluscs seems to be closely connected with calcium metabolism, and indeed Turner (1937) associated the formation of uroporphyrin I which occurs in the bones of the Pennsylvanian fox-squirrel (*Sciurus niger*), with the megaloblasts of the bone marrow. It is significant that it is the black variety of the slug, *Arion ater* in which the greatest amount of uroporphyrin I occurs (Kennedy, 1958), the amount of porphyrin being closely parallel to the amount of melanin in the integument, so that in the very pale grey specimens, no uroporphyrin I is detectable. *Sciurus niger* has black hair, so that in these two widely differing animals an additional pigment is present, namely melanin, which protects these animals from the photosensitizing effects of the porphyrin. *Aplysia* and *Akera* which are more darkly coloured, have more uroporphyrin I than *Duvaucelia* which has a bright orange pigment; there was a much greater concentration of coproporphyrin III in the body wall of the dark coloured *Arenicola* and *Amphitrite* than in the lighter, pink specimens: the more darkly pigmented *Asterias* contained more protoporphyrin IX than the paler animals (Kennedy & Vevers, 1953) although protoporphyrin is not noteworthy for its photosensitizing action. Fischer & Zerweck (1924) have shown in mammals that photosensitivity to u.v. light increases with the number of carboxylic groups in the porphyrin molecule, the action of proto-, deutero- and mesoporphyrin is very slight, but copro- and uroporphyrin have an intense photosensitizing action. The exception to this is haematoporphyrin which has not been detected free in nature so far, which has the greatest photosensitizing effect of all, and which has two carboxyl groups and two hydroxyethyl groups.

CONCLUSIONS

These results suggest that the heart-bodies (when present) in polychaetes are haematopoietic tissues.

Types of porphyria comparable with those described in man occur in invertebrates, and this may explain the apparently random distribution of free porphyrins in marine animals. Further support is given to the view that porphyrin metabolism and calcium metabolism are closely associated. Since all animals in which photosensitizing porphyrins occur also have dark pigments (frequently melanin, and sometimes carotenoid) the latter presumably have a protective function.

The ability of the heart-body and other polychaete tissues to form porphyrins from added PBG and δ-aminolaevulinic acid (ALA) will be the subject of a further investigation. It is of the first importance also to determine whether the heart-bodies contain PBGase. These experiments may lead to an understanding of the formation in the heart-bodies of the coproporphyrin III in such large amounts.

It is also of interest to note here that the suggestion of Picton (1898) that the heart-body is formed from an infolding of extravasal epithelia, is amply borne out by the equivalence, as shown by the porphyrins present, of the heart-bodies with the extravasal tissue in *Arenicola*, tissue frequently referred to by previous authors as 'chloragogen' (Romieu, 1923). It thus seems possible that such extravasal 'chloragogen' in polychaetes fulfils an haematopoietic role, without excluding the possibility that it participates in excretion or food storage as well (Dales, 1957). Romieu (1923) regarded the results of his histochemical and histological investigations as providing evidence for regarding the heart-body as a 'storage kidney', or intravascular 'chloragogue'. It is also intended to investigate this further. Eisig (1887) considered that the brown or brown-green pigment of the heart-body in *Terebella lapidaria* was a derivative of haemoglobin. Romieu (1923) did not agree with this, and did not elicit the reactions for haematin or biliverdin; he was of the opinion that this brown pigment was a 'urochrome', and compared it with chaetopterin, which he said was a modified chlorophyll, the comparison resting solely on its solubility in acetic acid and on its colour. Kennedy & Nicol (1958) have shown that the pigment hitherto known as chaetopterin is a mixture of phaeophorbides *a* and *b*, and as such could in fact be regarded as a modified chlorophyll. Experiments described in this present paper confirm Eisig's view that the brown granules in the heart-body (and also in the extravasal tissue of *Arenicola*) are a derivative of haemoglobin, in fact, haematin.

SUMMARY

The heart-bodies and some other tissues of a number of polychaetes have been examined chemically, and the porphyrin pigments from these tissues described. Coproporphyrin III was shown to be present in relatively large concentration in all the heart-bodies, together with traces of coproporphyrin I, a tricarboxylic porphyrin and protohaematin. The body wall of light (pink) and dark (brown) coloured *Arenicola* and of *Amphitrite* also contained these pigments in varying amounts. Other non-porphyrin pigments were also seen in the body wall of some animals, the most remarkable being a colourless compound with a bright blue-fluorescence occurring in *Flabelligera*. Evidence is presented which supports the view that the heart-body is an haematopoietic organ, and the significance of this is discussed. Parallels are drawn between the occurrence of free porphyrins in marine animals and the occurrence of such pigments in porphyrias in man.

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LUMINESCENCE IN POLYNOIDS

IV. MEASUREMENTS OF LIGHT INTENSITY

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(Text-figs. 1–3)

INTRODUCTION

Many polynoid worms (family Aphroditidae) are luminescent. In recent papers, physiological and histological aspects of polynoid luminescence have been considered in some detail (Bonhomme, 1942; Nicol, 1953, 1954, 1957*a, b*). The light appears in the scales or elytra which cover the dorsal surface of the worm. The photocytes form a single epithelial layer on the lower surface of the elytrum. They are concentrated near the centre of the scale and, when the latter is pigmented, there is a clear area above the photogenic tissue through which the light escapes.

Luminescence in polynoids is under nervous control and is evoked by external stimulation. The elytra usually flash repetitively to a single stimulus, and the responses can be analysed by recording from single elytra. Flash duration ranges from 100 to 200 msec. There is much variation in flash intensity: initially, consecutive flashes become progressively brighter, owing to facilitation, but fatigue soon sets in and the flashes become fainter and finally die away.

The light of polynoids is emerald-green in colour. Spectral emission extends from about 450 to 680 mμ, with a maximum at about 515 mμ (Nicol, 1957*c*). The spectral emission curve is shown in Fig. 1. This information has been utilized to calculate the intensity of polynoid light, in the manner now to be described.

MATERIAL AND METHODS

The light of two species was measured, viz. *Lagisca extenuata* and *Acholoë astericola*. Elytra were removed from the animals under $MgCl_2$-narcosis, and were subsequently washed in sea water. A single elytrum was mounted in a Perspex chamber over a pair of silver electrodes (see Nicol, 1953). Flashing was induced by stimulating with electric shocks: these were square wave pulses, about 2 msec in duration, and up to 10 V in intensity.

The light was detected by a photomultiplier (E.M.I. type no. 6685), the spectral sensitivity of which had been determined by the National Physical Laboratory. The photocathode of the photomultiplier is 9 mm in diameter. Sensitivity is maximal in the violet, and falls off steadily at long wavelengths, above 500 mμ (Fig. 2, curve A). The elytrum was positioned 2 cm beneath the photomultiplier, so that its upper surface lay beneath the centre of the photocathode, and was parallel to the face of the latter. The light-emitting region of the elytrum is less than 1 mm² in area, and can be regarded as a point-source.

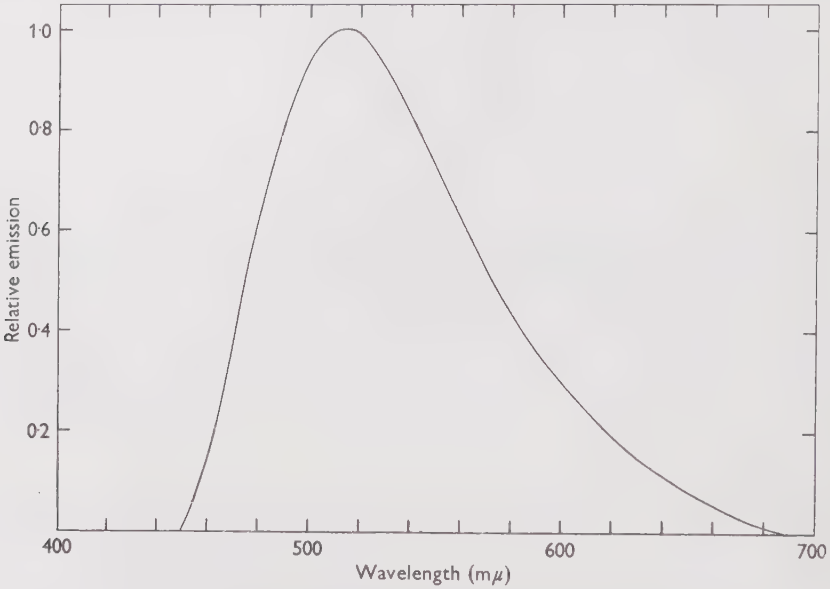

Fig. 1. Relative spectral emission curve of polynoid light (from Nicol, 1957*c*).

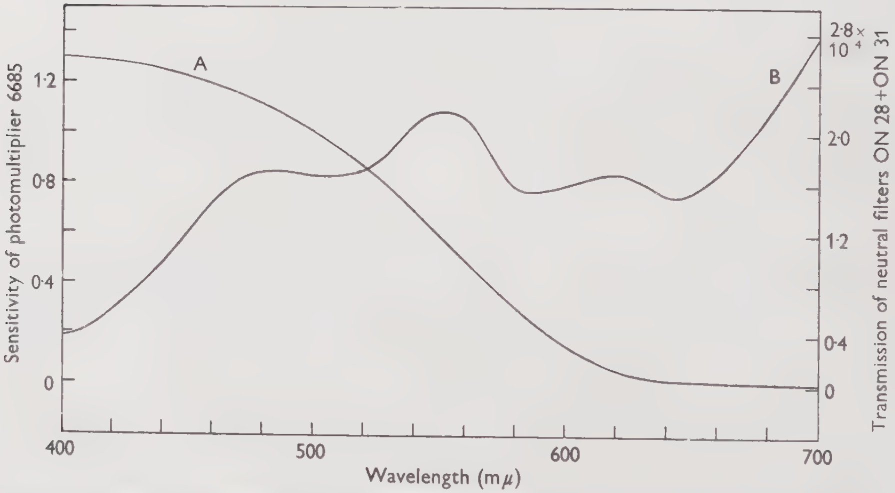

Fig. 2. Curve A: relative spectral sensitivity of photomultiplier type E.M.I. no. 6685. Curve B: combined transmission of two neutral glass filters (Chance ON 28 + ON 31).

The photomultiplier was connected to a cathode-ray oscilloscope, and photographic records of the responses were made on moving paper.

Measurements were made at ambient temperatures of 18–19° C.

CALIBRATION OF THE PHOTOMULTIPLIER

The sensitivity of the apparatus, that is of photomultiplier 6685 plus amplifier and C.R.O., was determined against a known light source. This was a substandard (tungsten) lamp, of colour temperature 2360° K, and energy output of 24·15 candelas. The lamp was calibrated by the National Physical Laboratory. It was placed on an optical bench 2 m from the face of the photomultiplier. Intensity was reduced by two neutral glass filters (Chance ON28+ON31), the transmission characteristics of which were measured in a spectrophotometer (Unicam SP500) (see curve B, Fig. 2).

The flux of light from the substandard lamp at 2 m, falling on the surface of the photomultiplier perpendicular to the direction of the light beam, is

$$\int_{400}^{700} pKJ_\lambda V_\lambda \,\mathrm{d}\lambda = pK\int_{400}^{700} J_\lambda V_\lambda \,\mathrm{d}\lambda = I/4\times 10^4 \text{ lumens/cm}^2.$$

I is the intensity of the substandard lamp in candelas, p is a quantity for the particular experimental set-up, and K is the luminous efficiency of radiation, which is 682 lumens/W at 555 mμ (Walsh, 1953):

$$p = \frac{I}{4\times 10^4}\times\frac{1}{682\int_{400}^{700} J_\lambda V_\lambda \,\mathrm{d}\lambda} \text{ W/cm}^2/\mathrm{m}\mu.$$

Let the light from the substandard lamp at 2 m, passing through the neutral filters, give a deflexion on the oscilloscope $= D_L$. Let T_λ be the combined transmission of the neutral filters, S_λ be the spectral sensitivity of the photomultiplier, and q be a constant such that $q/S_\lambda = \mathrm{W/cm}^2$ of a given wavelength (λ) required to produce unit deflexion. Then

$$D_L = \int_{400}^{700}\frac{pJ_\lambda S_\lambda T_\lambda \,\mathrm{d}\lambda}{q} = \frac{p}{q}\int_{400}^{700} J_\lambda S_\lambda T_\lambda \,\mathrm{d}\lambda$$

and

$$q = \frac{p}{D_L}\int_{400}^{700} J_\lambda S_\lambda T_\lambda \,\mathrm{d}\lambda \text{ W/cm}^2.$$

Values for V_λ were taken from the C.I.E. table for photopic vision (International Relative Luminous Efficiency of Radiation for Photopic Vision, table VI in Keitz, 1955). Values for J_λ were obtained from Skogland, 1929, for a lamp of colour temperature 2360° ($J=1$ at $\lambda = 590$ mμ).

To determine p and q, the following values were used. Intensity, I, of the substandard lamp is 24·15 candelas. With set amplification on the C.R.O.

(30 V/mm), and known voltage on the photomultiplier (1400 V), the deflexion produced by the light is 22·02 mm.

From this quantity, an estimate was made of the deflexion which would be produced at higher amplification, viz. 10 V/mm on the C.R.O., and photomultiplier voltage of 1600 V. This estimated deflexion was 227·36 mm.

$$\int_{400}^{700} J_\lambda V_\lambda = 83{\cdot}52,$$

and $$p = \frac{24{\cdot}15}{4 \times 10^4} \times \frac{1}{682 \times 83{\cdot}52} = 1{\cdot}0599 \times 10^{-8} \text{ W/cm}^2/\text{m}\mu.$$

$$\int_{400}^{700} J_\lambda S_\lambda T_\lambda \, d\lambda = 105{\cdot}1 \times 10^{-4}$$

and $$q = \frac{1{\cdot}0599 \times 10^{-8}}{227{\cdot}36} \times 105{\cdot}1 \times 10^{-4} = 0{\cdot}49 \times 10^{-12} \text{ W/cm}^2.$$

In order to make periodical checks on the sensitivity of the apparatus, an alternative light source was employed. This consisted of a stilbene phosphor irradiated by ^{60}Co (called, for brevity, Co source). The phosphor emitted a very faint blue light. Random emission by the phosphor gave a very broad beam-trace, which was smoothed out by putting a 0·1 μF condenser across the input of the oscilloscope. The Co source was set 2 cm from the face of the photomultiplier, and the oscilloscope deflexion was photographed.

MEASUREMENTS OF THE INTENSITY OF POLYNOID LIGHT

Records were obtained of the flashes of eight elytra of *Lagisca*, and of six elytra of *Acholoë*. Some examples are shown in Fig. 3. Various amplifications were used, and the measured deflexions were calculated on the basis of instrument-sensitivity occurring at a C.R.O. setting of 10 V/mm and 1600 V on the phototube. These calculated deflexions, D_s, for elytra at 2 cm distance, are shown in columns 2 of Tables 1 and 2. Column 3 shows calculated deflexions at a distance of 1 m, according to the inverse square law.

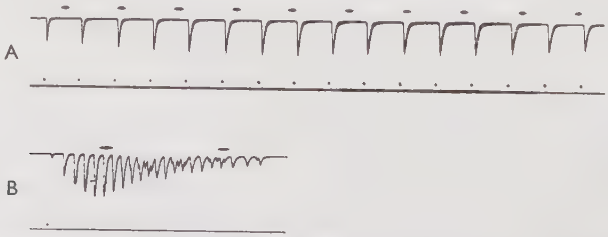

Fig. 3. Oscillograph records of the flashing of polynoid elytra. A, *Acholoë*, B, *Lagisca*. Time signal, above, 1/sec. Electrical stimuli on lower line.

The flashes of polynoids are much briefer than 1 sec. In order to calculate the energy of one flash, the response curve for a flash was averaged over 1 sec in terms of a maximal deflexion lasting 1 sec. These calculations gave factors, A, for response curves of: 0·02 for *Lagisca*, and 0·084 for *Acholoë*. The deflexions (D_s) for the responses of *Lagisca* and *Acholoë*, averaged over 1 sec, are shown in columns 4 of Tables 1 and 2 ($D = D_s A$).

TABLE 1. CALCULATION OF THE INTENSITY OF LIGHT EMITTED BY AN ELYTRUM OF *LAGISCA*

Elytrum and record	Maximal deflexion D_s at 2 cm (mm)	Calculated deflexion D_s at 1 m (mm)	D $(= A^{\star} \times D_s)$ at 1 m	Energy at 515 mμ in 1 flash, μJ/cm² at 1 m $(\times 10^{-12})$	Total energy in 1 flash, μJ/cm² at 1 m $(\times 10^{-10})$
2A	0·6	0·00024	0·0000048	0·03129	0·03309
B	0·9	0·00036	0·0000072	0·046944	0·04964
C	0·3	0·00012	0·0000024	0·015648	0·01655
D	0·55	0·00022	0·0000044	0·028688	0·03034
3A	4·1	0·00164	0·0000328	0·213856	0·22615
B	2·5	0·00100	0·0000200	0·13040	0·13790
C	16·5	0·00660	0·0001320	0·860640	0·91013
4A	0·9	0·00036	0·0000072	0·046944	0·04964
B	4·5	0·00180	0·0000360	0·234720	0·24822
C	52·5	0·02100	0·0004200	2·73840	2·89586
5A	6	0·00240	0·0000480	0·31296	0·33096
B	3·9	0·00156	0·0000312	0·203424	0·21512
6A	3	0·00120	0·0000240	0·15648	0·16548
B	66	0·02640	0·0005280	3·44256	3·64051
7A	10·8	0·00432	0·0000864	0·56332	0·59572
B	43·2	0·01728	0·0003456	2·253312	2·38286
8	6·48	0·002592	0·0000518	0·337997	0·35743
9A	8·64	0·003456	0·0000691	0·450662	0·47658
B	1·44	0·000576	0·0000115	0·075110	0·07943

$$\star\ A = \frac{\text{area of response curve for a single flash averaged over 1 sec}}{\text{maximal response} \times 1\text{ sec}}.$$

TABLE 2. CALCULATION OF THE INTENSITY OF LIGHT EMITTED BY AN ELYTRUM OF *ACHOLOË*

Elytrum and record	Maximal deflexion D_s at 2 cm (mm)	Calculated deflexion D_s at 1 m $(\times 10^{-4})$ (mm)	D $(= A^{\star} \times D_s$ at 1 m) $(\times 10^{-4})$	Energy at 515 mμ in 1 flash, μJ/cm² at 1 m $(\times 10^{-10})$	Total energy in 1 flash, μJ/cm² at 1 m $(\times 10^{-8})$
1A	1·824	7·296	0·6129	0·39961	0·42259
B	8·683	34·732	2·9175	1·90221	2·01159
2	0·917	3·668	0·3081	0·20089	0·21244
5A	1·643	6·572	0·5520	0·35994	0·38064
B	7·126	28·504	2·3943	1·56108	1·65084
8	1·376	5·504	0·4624	0·30148	0·31882
9A	1·099	4·396	0·3693	0·24078	0·25462
B	1·985	7·940	0·6670	0·43488	0·45989

★ See Table 1 for definition.

A relative spectral emission curve for polynoid light is shown in Fig. 1, in which relative energy, E_λ, is plotted against λ (Nicol, 1957*c*). This curve can be put on an absolute basis in terms of a quantity r such that $E_\lambda r$ gives Watts/cm² of receptor surface/mμ under the experimental conditions specified.

$$D = \int \frac{rE_\lambda S_\lambda}{q}\,\mathrm{d}\lambda, \qquad D = \frac{r}{q}\int E_\lambda S_\lambda\,\mathrm{d}\lambda.$$

$$r = \frac{Dq}{\int E_\lambda S_\lambda\,\mathrm{d}\lambda}\ \mathrm{W/cm^2/m\mu}, \qquad \int_{400}^{700} E_\lambda S_\lambda\,\mathrm{d}\lambda = 75{\cdot}1,$$

$$r = D \times \frac{0{\cdot}49 \times 10^{-12}}{75{\cdot}1} = D \times 0{\cdot}652 \times 10^{-14}\ \mathrm{W/cm^2/m\mu}.$$

The energy at 515 mμ in 1 flash is equal to

$$r = D \times 0{\cdot}652 \times 10^{-14}\ \mathrm{W/cm^2/m\mu}\ \text{at 1 m.}$$

The total energy in 1 flash is equal to

$$r\int_{400}^{700} E_\lambda\,\mathrm{d}\lambda = D \times 0{\cdot}652 \times 10^{-14} \int_{400}^{700} E_\lambda\,\mathrm{d}\lambda\ \mathrm{W/cm^2}\ \text{at 1 m.}$$

From integration of the curve in Fig. 1,

$$E_\lambda\,\mathrm{d}\lambda = 105{\cdot}75.$$

Values for energy in 1 flash at 515 mμ, and for total energy in 1 flash, are shown in columns 5 and 6 of Tables 1 and 2. For *Lagisca* the total energy in a flash ranges from $0{\cdot}017 \times 10^{-10}$ to $3{\cdot}641 \times 10^{-10}$ μJ/cm² at 1 m. For *Acholoë*, the total energy in a flash ranges from $0{\cdot}212 \times 10^{-8}$ to $2{\cdot}012 \times 10^{-8}$μ J/cm² at 1 m.

These values were determined by measuring that light which was emitted in a cone having its axis perpendicular to the upper surface of the elytrum, and possessing a solid angle $w = 0{\cdot}16$ sterad.

The data and calculations of Tables 1 and 2 pertain to a single flash. Polynoid elytra usually flash repetitively, so that there may be 1 or many flashes in a given second; at fast rates there is often some degree of summation. A calculation for *Lagisca* gives the following results:

(*a*) Total energy in a single brief flash (maximal flash in a series of flashes): $0{\cdot}182 \times 10^{-9}$ μJ/cm² at 1 m.

(*b*) Total flux in a series of 13 flashes occurring in 1 sec (maximal flash intensity as in (*a*)): $2{\cdot}600 \times 10^{-9}$ μW/cm² at 1 m.

INTENSITY OF LIGHT EMITTED PER PHOTOCYTE

The photocytes lie in a single cellular layer. This arrangement permits an estimation of the light emitted per photocyte. Elytra were sectioned (at 7 μ), and the total number of photocytes counted. In elytrum no. 7 of *Lagisca* there were 1250 photocytes. Minimal and maximal values for the light emitted by a photocyte in 1 flash are: minimum, $0{\cdot}048 \times 10^{-12}$ μJ/cm^2 at 1 m; maximum, $0{\cdot}191 \times 10^{-12}$ μJ/cm^2 at 1 m.

COMMENT

Mean values for the intensity of light emitted in a single flash by an elytrum of *Lagisca* are $1{\cdot}8 \times 10^{-6}$ μJ/cm^2 at 1 cm, and $1{\cdot}8 \times 10^{-10}$ μJ/cm^2 at 1 m. Corresponding values for the flash of an elytrum of *Acholoë* are $1{\cdot}11 \times 10^{-4}$ μJ/cm^2 at 1 cm and $1{\cdot}11 \times 10^{-8}$ μJ/cm^2 at 1 m. These estimates are for air-paths.

Some estimations are available for the radiant flux emitted by other marine species. The luminescence of a ctenophore *Mnemiopsis leidyi*, 35 mm in diameter, was $0{\cdot}5 \times 10^{-4}$ to $>0{\cdot}75 \times 10^{-4}$ μW/cm^2 at 50 cm (recalculated as $0{\cdot}125 \times 10^{-4}$ to $>0{\cdot}187 \times 10^{-4}$ μW/cm^2 at 1 m in air) (Clarke & Backus, 1956). The light of *Euphausia pacifica* has a mean intensity of $1{\cdot}8 \times 10^{-3}$ μW/cm^2 at 1 cm. An adult *Euphausia pacifica* is about 20 mm long and has 10 light-emitting organs (photophores). The light intensity of *Pyrosoma atlantica* ranges from 8×10^{-3} to 4×10^{-2} μW/cm^2 at 1 cm. A colony 10 cm long emitted light of intensity $2{\cdot}5 \times 10^{-2}$ μW/cm^2 at 1 cm (Kampa & Boden, 1957).

The flashes of polynoids are shorter than 1 sec. If the light of these worms remained constant during 1 sec at maximal flash intensity, then the radiant flux for an elytrum of *Lagisca* and *Acholoë*, respectively, would be: $0{\cdot}9 \times 10^{-4}$ and $1{\cdot}31 \times 10^{-3}$ μW/cm^2 at 1 cm (in air). These values for radiant flux of polynoid elytra are of about the same order as that for *Euphausia*. Ctenophores and Pyrosomas are much bigger animals, with correspondingly larger light-emitting surfaces.

The light emitted in 1 flash by a single photocyte of *Lagisca* is $0{\cdot}12 \times 10^{-12}$ μJ/cm^2 receptor surface at 1 m. For a flash of 1 sec duration, the estimated radiant flux would be $1{\cdot}3 \times 10^{-12}$ μW/cm^2 receptor surface at 1 m. Harvey (1925) has estimated the total radiant flux emitted by a single bacterium of *Bacillus phosphorescens* to be $4{\cdot}95 \times 10^{-10}$ μW at $\lambda = 510$ mμ (the wavelength of maximal emission). Over the entire emission spectrum $\left(\text{i.e. } \int_{400}^{700} E_\lambda \, d\lambda\right)$, the radiant flux emitted by a single bacterium is about $0{\cdot}4 \times 10^{-12}$ μW/cm^2 receptor surface at 1 m. I have recently estimated the energy in a single brief flash from a cell of *Noctiluca miliaris* to be about $1{\cdot}1 \times 10^{-12}$ μJ/cm^2 receptor surface at 1 m (in air) (unpublished research). This is about ten times greater than the light output of a photocyte of *Lagisca*.

The threshold of the dark-adapted human eye for a flash having a duration $<0{\cdot}1$ sec is about 100 quanta at 510 mμ in a test field of 10′ (Graham & Margaria, 1935; Hecht, Shlaer, & Pirenne, 1942; Pirenne & Denton, 1952). When fully exposed, the human pupil has an area, mean $S = 0{\cdot}5$ cm². Now, 1 quantum at 510 $\text{m}\mu = 4 \times 10^{-12}$ erg. For a just detectable flash, the human eye needs 100 quanta $= 4 \times 10^{-10}$ erg to fall in the pupil, area S.

The mean value for energy in a flash of *Lagisca* is $1{\cdot}8 \times 10^{-10}$ μJ/cm² receptor surface at 1 m, or $1{\cdot}8 \times 10^{-9}$ erg/cm² receptor surface at 1 m.

At x m, the equivalent energy, if all were concentrated at 510 mμ, in a polynoid flash

$$= \frac{1{\cdot}1 \times 10^{-9}}{x^2} \text{ erg/cm}^2 \text{ receptor surface}$$

$$= \frac{1{\cdot}1 \times 10^{-9}}{x^2} S \text{ erg/mean pupil area.}$$

The distance in air at which an average flash of *Lagisca* can be seen, by the dark-adapted human eye, is given by

$$4 \times 10^{-10} \text{ erg} = \frac{1{\cdot}1 \times 10^{-9}}{x^2} S \text{ erg/mean pupil area,}$$

$$x^2 = \frac{1{\cdot}1 \times 10^{-9} \times 0{\cdot}5}{4 \times 10^{-10}} \text{ metres squared,}$$

$$x = 1{\cdot}2 \text{ m.}$$

I am indebted to Dr E. J. Denton for guidance in the calculations of light intensity. Part of the apparatus was purchased with grants from the Royal Society. The ^{60}Co-stilbene light source was devised and loaned by Dr G. N. Harding of the U.K. Atomic Energy Authority, to whom I am grateful.

SUMMARY

The light energy emitted in a flash by single elytra of two polynoid worms has been measured, viz. *Acholoë astericola* and *Lagisca extenuata*. Maximal emission occurs at 515 mμ. Mean values for light intensity per flash from 1 elytrum are: *Lagisca*, $1{\cdot}8 \times 10^{-10}$ μJ/cm² receptor surface at 1 m; *Acholoë*, $1{\cdot}11 \times 10^{-8}$ μJ/cm² receptor surface at 1 m. The light emitted in 1 flash by a single photocyte of *Lagisca* is $0{\cdot}12 \times 10^{-12}$ μJ/cm² receptor surface at 1 m. It is estimated that the light from 1 elytrum of *Lagisca* could be seen by the dark-adapted human eye at 1·2 m in air.

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SPECTRAL COMPOSITION OF THE LIGHT OF *PHOLAS DACTYLUS* L.

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(Text-figs. 1–6)

The light of the piddock *Pholas dactylus* L. appears bluish, but its spectral energy composition has not been determined. A spectral energy curve for the light of this species is desirable in order to calculate absolute energy content (radiant flux) and to relate it to pertinent visibility curves. To this end I have determined relative spectral energy approximately by means of a set of nine different spectral filters, covering the visual spectrum and the near ultra-violet and infra-red.

MATERIAL AND METHODS

Specimens of *Pholas dactylus* were obtained from the red sandstone at Exmouth, near low tide level. The animals were anaesthetized with iso-osmotic $MgCl_2$ and the luminous regions removed. These have been described and figured by Panceri and others (literature in Harvey, 1952). I selected the paired triangular organs in the mantle cavity and the siphonal cords for recording. The bits of tissue were washed in running sea water, and pinned out in black dishes.

The light from the pholads was analysed by means of spectral filters mounted in a rotating disc. The luminescent tissue was placed below the disc, the photomultiplier above, so that light passed through the various filters in turn as the disc revolved. Rotation of the disc was slow, about 1 revolution each 3 min. The photomultiplier was connected to a cathode-ray oscilloscope, and the deflexions were photographed on moving paper. Noise level was reduced by placing a 0·05 μF condenser across the input of the oscilloscope. The apparatus is described in more detail elsewhere (Nicol, 1957).

The photomultiplier was an E.M.I. tube, type no. 6685, with a blue-sensitive photocathode, the spectral sensitivity of which had been measured by the National Physical Laboratory. The disc contained the same spectral filters as the disc IV used for *Chaetopterus* (Nicol, 1957, p. 631), namely, in sequence, Ilford red 608, Ilford orange 607, Ilford yellow 606, Ilford yellow-green 605, Ilford blue-green 603 plus Chance neutral ON31, Ilford blue 602 plus Chance neutral ON32, Ilford violet 601 plus Chance neutral ON33, Chance deep purple OV1, with Ilford green 604 interposed between each.

Curves for the products of sensitivity of the photomultiplier (S_λ) times transmission of a filter (T_λ), plotted against λ, are given in Nicol (1957, fig. 5). In the same paper will be found values for $\eta_x = \int S_\lambda T_\lambda d\lambda$, and mean representative wavelengths (mean λ) for these particular filters (x = filter).

Luminescence was evoked by electrical stimulation. Light is extracellular, and the response is slow, lasting several minutes. Temperatures were 18–19° C.

OBSERVATIONS

A photographic record of the responses obtained with the spectral filters is shown in Fig. 1. Responses obtained with the green filters No. 604 were used as reference levels. Let $D_{604(1)}$ and $D_{604(2)}$ be the measured deflexions of two contiguous green filters 604, and let D_x be the measured deflexion of a spectral filter placed between the two green filters. Then the corrected response is $2D_x/(D_{604(1)}+D_{604(2)}) = R_x$. By this means, changes of light-intensity at the source are compensated for.

First approximate results $R_x/\eta_x = Q_x$ are plotted in Fig. 2. The light is blue-green, with a peak at about 490 mμ. There is no emission below 410 mμ, and emission in the red is small.

To correct these first results for broad spectral transmission of the filters, the following procedure was adopted. Spectral energy levels ($E_{\lambda A}$) were taken from the curve in Fig. 2, and new curves were drawn for $E_{\lambda A} S_\lambda T_\lambda$ against λ (Fig. 4). Ratios were calculated:

$$\rho_1 = \frac{\int E_{\lambda A} S_\lambda T_\lambda \text{ filter } X}{\int E_{\lambda A} S_\lambda T_\lambda \text{ filter 603}}, \quad \rho_2 = \frac{R_x}{R_{603}},$$

and

$$\rho_2/\rho_1 = \rho_3.$$

The values for ρ_3 were then used to correct the first approximate results, thus $Q_x \rho_3$. Values for the latter are plotted against mean λ in Fig. 3, and a new

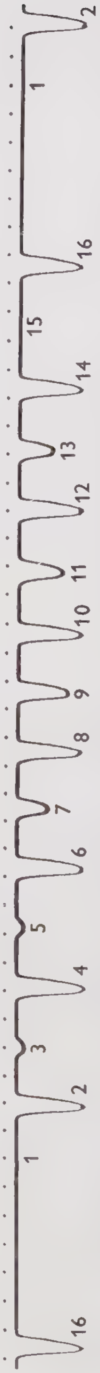

Fig. 1. Oscillograph record of the light of *Pholas* passed through spectral filters. Filters presented in the following order: 1, red 608; 2, green 604; 3, orange 607; 4, green 604; 5, yellow 606; 6, green 604; 7, yellow-green 605; 8, green 604; 9, blue-green 603+Chance neutral ON31; 10, green 604; 11, blue 602+Chance neutral ON32; 12, green 604; 13, violet 601+Chance neutral ON33; 14, green 604; 15, Chance deep purple OV1; 16, green 604. Light falling on the photomultiplier causes a downward deflexion of the lower trace. Time scale above ⅛ sec.

spectral energy curve is drawn for these values. The value of mean λ for each filter, used in Fig. 3, has been estimated from the centre of gravity of the individual curves $E_{\lambda A}S_{\lambda}T_{\lambda}$ of Fig. 4.

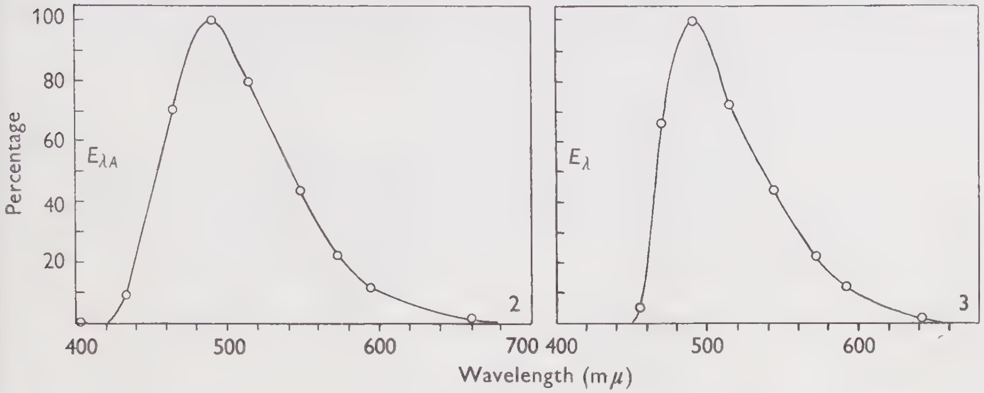

Fig. 2

Fig. 3

Figs. 2, 3. Spectral composition of the light of *Pholas dactylus*. Relative emission plotted against mean λ of the filters. Fig. 2, first approximate results. Fig. 3, corrected results (see text for details).

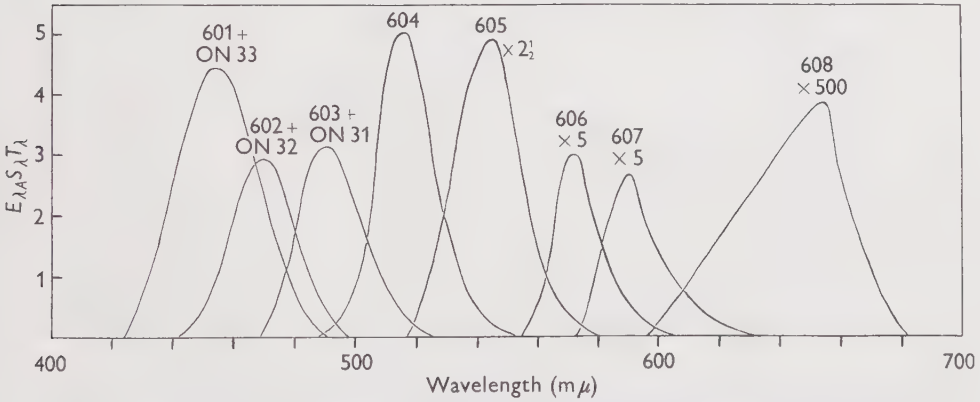

Fig 4. Plots of $E_{\lambda A}S_{\lambda}T_{\lambda}$ against λ. ($E_{\lambda A}$ = relative spectral emission (from Fig. 2); S_{λ} = relative spectral sensitivity of photomultiplier; T_{λ} = transmission of filter(s).)

COMMENT

The light of *Pholas* is blue-green. Spectral emission extends from about 440 to 670 mμ, with a maximum at about 490 mμ. The action spectrum of *Pholas* has been determined (Hecht, 1928), and it is reproduced in Fig. 5. This has a maximum at about 545 mμ, with evidence for another rise in the violet. Visibility and luminescence spectra, therefore, are quite dissimilar in shape, although all energy is emitted in a spectral range to which *Pholas* is sensitive. 'Luminous flux' for *Pholas* vision and human scotopic vision have been calculated, and curves for these values plotted against λ are shown in Fig. 6. 'Luminous efficiencies' (total luminous flux ÷ total radiant flux) are

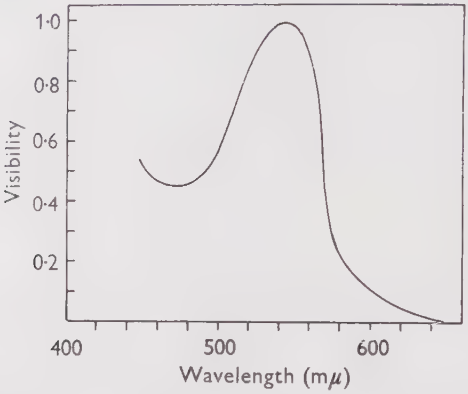

Fig. 5. Action spectrum of the light of *Pholas dactylus*. From Hecht, 1928.

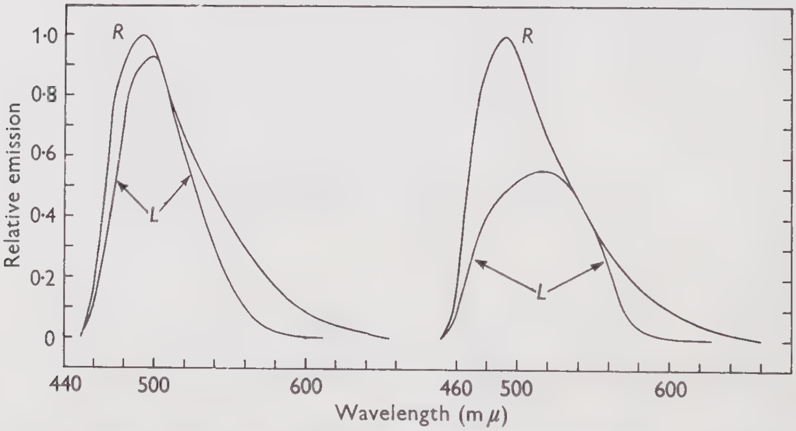

Fig. 6. Comparison of radiant flux (R) of *Pholas* light with luminous flux (L). Left, luminous flux based on human scotopic vision. Right, luminous flux based on Hecht's visibility curve for *Pholas*.

61% for *Pholas* visibility, and 76% for human scotopic vision. The use to which *Pholas* puts its light is unknown. Absolute threshold for photosensitivity and the intensity of *Pholas* luminescence have not yet been determined, so it is uncertain whether *Pholas* can detect or respond to its own light.

SUMMARY

A spectral emission curve for the light of *Pholas dactylus* has been determined by means of spectral filters and photomultiplier cell. Emission extends from about 440 to 670 mμ, with a maximum at about 490 mμ. The emission curve is compared with the action spectrum determined by Hecht.

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NOTE ON THE TEMPERATURE TOLERANCES OF SOME INTERTIDAL ANIMALS IN RELATION TO ENVIRONMENTAL TEMPERATURES AND GEOGRAPHICAL DISTRIBUTION

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(Text-figs. 1–3)

It is generally acknowledged that temperature plays a large part in controlling the distribution of animals (see, for example, chapter 6 in Andrewartha & Birch, 1954). Often, however, other environmental factors may conceal or modify the influence of temperature. On the seashore, analysis of temperature effects is complicated by the presence of many other variables, such as those associated with the daily rhythms of emergence and submergence; it is particularly difficult to assess the relative importance of the temperature of the sea and of the air. Nevertheless, previous attempts have been made to correlate the distribution of some intertidal animals with temperature, the particular value of mean, extreme, seasonal or annual sea or air temperature chosen being that which fits best the distribution of the animal in question (e.g. Hutchins, 1947; Southward, 1950; Southward & Crisp, 1954). Unfortunately, there is little direct evidence for a causal relation between temperature and distribution. Some observations and experiments have been made on the influence of temperature on the breeding and development of a few marine animals (e.g. Orton, 1920; Runnström, 1929; Loosanoff, Miller & Smith, 1951), but until recently little was known of the temperatures that could be tolerated, especially in the adult stage. It is known that the adult stage of some marine animals may tolerate a wider range of temperature than the egg or larva (e.g. Vernon, 1899), but clearly the adult stage of an intertidal animal will be exposed to more extreme temperatures (effect of air temperature, sunlight, frost) than the planktonic larvae, or the egg masses developing in large or deep tide pools and the shelter of crevices and sea weed.

The experiments that have been carried out on the resistance of intertidal animals to temperature extremes have usually been designed to show the influence of high temperature on their vertical zonation (Huntsman & Sparks, 1924; Gowanloch & Hayes, 1926; Broekhuysen, 1940; Evans, 1948), or the effect of low temperature on ice-formation in the tissues (Kanwisher, 1955). It is therefore worth presenting the results of some laboratory experiments that were carried out to investigate the effect of temperature extremes on the adults of eight species of intertidal animals and thus determine what influence, if any, extremes of temperature might have in nature on the distribution of the adult stage. The experiments were made concurrently with field investigations on the distribution of the same and other species, reported in part elsewhere in this Journal (Crisp & Southward, 1958, p. 157). In addition to laboratory experiments, attempts were made to assess temperatures in the intertidal zone, as distinct from sea or air temperatures, by measuring the tissue temperatures of some of the animals under various weather conditions on the shore.

The results of the work on temperature tolerance and on temperatures in the intertidal zone are presented separately below, and then discussed together in relation to the geographical distribution of the animals.

All temperatures are quoted in degrees Centigrade.

TEMPERATURE TOLERANCE

The temperatures tolerated by an animal fall into three successively wider ranges. The narrowest range of temperature is that over which the animal feeds, moves about and shows normal respiratory movements or behaviour; a somewhat wider range of temperature is tolerated without loss of irritability, though the animal may not carry out any spontaneous activity; an even wider range of temperature can be borne before the animal dies (Fig. 1). The

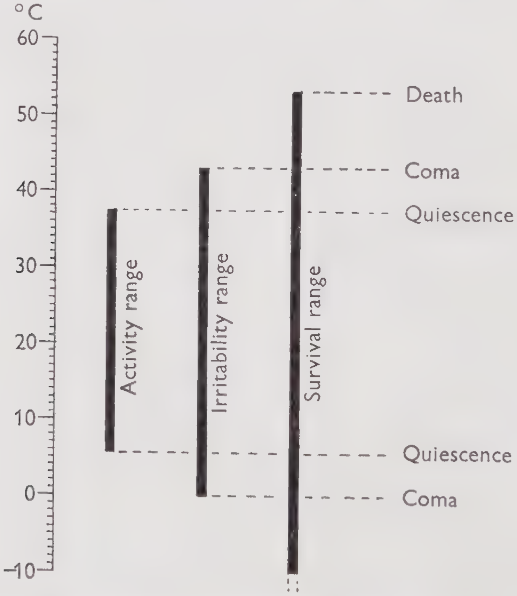

Fig. 1. Example of the three ranges of temperature tolerance of an animal. The barnacle *Chthamalus stellatus*; details of activity range and irritability range taken from Southward (1955). The lower death limit has not been ascertained.

temperature at which normal activity ceases may be termed the quiescence point, while the temperature at which irritability is temporarily lost is usually termed the point of coma. For the present purposes emphasis was placed on survival rather than ability to carry on normal activities, and the widest range of temperature tolerated has been determined. Some observations were made on the temperature limits for normal activity in a few species, but the results have been reported elsewhere (Southward, 1955, 1957).

There is no general agreement on the criteria used to determine lethal temperatures. I have followed Evans (1948) in using the same criterion for each species, namely the failure to respond to pricking with a needle within 12 h of return to normal temperature. Some investigators have used different criteria for each species (Orr, 1955), a procedure which may be justified where animals of very different habitat or habit are being compared. There is further disagreement over the proportion of a sample that need be killed to provide an estimate of the lethal point of the species or population. It has sometimes been said that total mortality of the sample has most biological meaning (cf. Edwards, 1946). If, however, a normal distribution of varying individual resistance to temperature is assumed it might be expected that a large sample would give a relatively wide range of tolerance before 100% mortality was reached, and it is more logical to take 50% mortality as an estimate of the population resistance. This criterion was used in the present work, but it was found that with the samples taken there was little difference between the 50 and 100% points (see Table 2, p. 55).

When the influence of duration on the resistance of an animal to extreme temperatures is being considered it is difficult to decide on the procedure to be adopted. Heilbrunn (1943) favours the construction of time-temperature curves showing the time taken to die at various temperatures, and Orr (1955) has worked on these lines. The latter, however, removed the animals directly from normal temperatures to high temperatures, without allowing time for conduction of heat to the internal organs. It is probably for this reason that the curves given (Orr, 1955) flatten out near the upper limits of temperature tolerance, the region between 0 and 10 min on the graph representing the time taken for equilibration between the tissues and the environment (compare Fig. 2 of this paper). The time taken to reach equilibrium must vary with each species. I have followed Huntsman & Sparks (1924), Broekhuysen (1940) and Evans (1948) in taking the animals in water at their normal temperature and heating them slowly. Apparatus limitations prevented a satisfactorily controlled slow cooling in the low-temperature experiments.

METHODS

The species of barnacles and molluscs used in the experiments are listed in Table 1, together with details of their normal zonation on the shore and their geographical distribution. As far as possible the animals were collected from the same area of shore for each set of experiments, and used within 1–2 days. The species that had to be collected from more distant shores, however, were sometimes kept under sea-water circulation in the laboratory for up to 2 weeks before use, and there were obvious chances of acclimatization to conditions different from those on the shore.

The experiments were carried out during the winter of 1953–4; it was not possible to repeat the experiments on all species during the following summer, as originally planned. However, the seasonal differences in high-temperature tolerance found in the species that were investigated were not significant in relation to the temperature intervals (0·5°) used.

TABLE 1. THE SPECIES INVESTIGATED

Species	Locality at which species were collected	Tide-level at which sp. taken	Normal zonation of species	Distributional status (from Crisp & Southward, 1958)
Barnacles				
Chthamalus stellatus (Poli)	Rum Bay and Wembury Beach	M.H.W.N.	Midlittoral, from M.H.W.S. to M.L.W.N.	Southern, not in North Sea or eastern Channel
Balanus balanoides (L.)	Brixham	M.T.L.	Midlittoral, from M.H.W.N. to M.L.W.N.	Northern
B. perforatus (Bruguière)	Wembury Beach	M.L.W.N.	Lower midlittoral, M.T.L. to M.L.W.S.	Southern, in S.W. England and S. Wales only
Elminius modestus Darwin	Hen Point	M.L.W.N.	Midlittoral and infralittoral M.H.W.N. to shallow water	Immigrant from Australasia
Top-shells				
Monodonta lineata (da Costa)	Rum Bay and Wembury Beach	M.H.W.N.	Midlittoral, M.H.W.S. to M.L.W.N.	Southern, not in Scotland, North Sea, or eastern Channel
Gibbula umbilicalis (da Costa)	Rum Bay and Wembury Beach	M.H.W.N.	Midlittoral, M.H.W.N. to M.L.W.N.	Southern, not in North Sea or eastern Channel
G. cineraria (L.)	Rum Bay and Wembury Beach	M.L.W.N.	Infralittoral, M.L.W.N. to shallow water	Northern
Calliostoma zizyphinum (L.)	Rum Bay and Wembury Beach	M.L.W.N.	Infralittoral, M.L.W.N. to shallow water	Northern

High temperature

In testing tolerance of high temperature the animals were taken at room temperature (15–20°) in a small vessel of water, and the temperature raised slowly by heating the vessel in a water bath. For the molluscs the rate of heating was adjusted to 1° per 5 min, a value adopted by previous workers. For the barnacles, which were smaller, a rate of heating of 1° per min was found to allow sufficient time for penetration; tests with thermocouples showed no lag greater than 0·1° between the water and the tissues.

It will be realized that these experiments represent rather unnatural conditions, for on the shore the animals would experience such high temperatures only out of the water, when they might be subjected to desiccation also. Since the experiments were designed to show purely temperature effects their unnatural character could not be avoided. It is possible that some species may be more resistant than others when drying effects are added to those of heat.

For what may be termed the instantaneous lethal points, batches of three to ten animals, the number depending on the physical size and abundance of the species, were removed from the vessel at certain temperature intervals, which were narrowed down to 0·5° in successive experiments, and allowed to recover in water at room temperature. The temperature at which more than half a batch failed to recover later within 12 h, and respond to pricking with a needle (by movement of the foot or valves), was regarded as the lethal temperature. When the instantaneous lethal point had been determined the effect of duration of high temperatures was studied. The temperature was raised as before and batches of animals removed at fixed temperatures (30°, 37°, 40° and 50°) to vessels of water maintained at the same temperature (plus or minus 1°) in an incubator; the fixed temperatures chosen for each species depending on the position of its instantaneous lethal point. Batches of animals were removed from the incubator at fixed intervals of time, allowed to recover in sea water at room temperature, and tested for recovery as before. For experiments of more than 1 day's duration the sea water in the incubators was made up with glass-distilled water to replace losses due to evaporation. In all experiments the water was aerated, and kept stirred, with compressed air.

Low temperature

The experiments on low temperature tolerance were hindered by difficulties over temperature control, and it was not possible to measure the instantaneous lethals. The animals were taken from water at room temperature, mopped dry on the outside of the shell with a clean cloth and placed in a dry finger-bowl covered by a sheet of glass. The finger-bowls were stacked in a small ice-cream conservator, set to 0°, −5° or −10° by means of a thermostat that was normally capable of controlling the temperature to plus or minus 1°. It was considered that under these circumstances heat transfer would be slow enough to prevent differences of tissue temperature between large and small animals, though the rate of change might be greater than that used for molluscs at high temperature.[1] Batches of animals were removed from the freezer at fixed intervals, and allowed to recover in sea water at room temperature. Those that failed to recover within 12 h and respond to pricking with a needle were considered dead. Some experiments were tried with the animals in sea water, but there were discrepancies between different sets, probably owing to the insulating properties of the ice that formed.

RESULTS

The results of the temperature tolerance experiments are given in Table 2. Clearly there was a much greater tolerance among the sessile barnacles than among the mobile top-shells, and the results for the two groups must be considered separately.

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[1] Relative humidity was approx. 50%.

Barnacles

Tolerance of high temperature in barnacles seems related both to the distribution of the species and to the normal habitat on the shore. Thus of the two southern forms, *Chthamalus stellatus*, which can occur at much higher levels on the shore, was more resistant at all temperatures, and was the only animal tested to show an instantaneous lethal temperature above 50°. The other southern species, *Balanus perforatus*, is not normally found above mid-tide level, and its temperature tolerance was little better than the northern species, *B. balanoides*, which can live at higher levels on the shore. The immigrant Australasian species, *Elminius modestus*, was more resistant to high temperatures than either native species of *Balanus*; it can sometimes occur at higher levels on the shore than either.

The barnacles were remarkably tolerant of low temperatures. All four species recovered after several weeks at zero, even though they experienced chill coma after an hour's exposure: it was not possible to determine the 50% lethal point in the time available. At lower temperatures, −5° and −10°, *B. perforatus* was much less tolerant than the others. There was little difference in resistance of the other species at −10°, but at −5° the immigrant species *Elminius* was least tolerant, while the northern form *Balanus balanoides* was nearly twice as resistant as *Chthamalus stellatus*. Most of these differences are in accord with the natural distribution of the species. The relatively low resistance of *Elminius* is surprising in view of its present abundance on the cold east coast of England, where the southern species do not occur. It is interesting to record that in another series of experiments a specimen of *Monodonta lineata* was found dead after 72 h exposure at −5°, with a dead *Elminius* and a living *Chthamalus* on its shell.

Top-shells

In the molluscs tested temperature tolerance was more clearly related to vertical distribution on the shore than to geographical distribution. Thus, the two southern forms, *Monodonta lineata* and *Gibbula umbilicalis*, which occur higher up the shore, were more tolerant of both high and low temperatures than the two northern forms, *G. cineraria* and *Calliostoma zizyphinum*, which occur only at low water. In fact, for all four species the temperature tolerances increased in proportion to the relative capacity to withstand exposure out of the water. At −10° little difference in resistance was found; the value of 2–3 h given in the table is probably the time required for equilibration between the tissues and the air at this low temperature. The relatively low resistance of the low-water species to temperatures below 0° and above 30° is interesting, but in view of the normal habitat of the species may not be very important.

TABLE 2. THE TEMPERATURE TOLERANCES OF FOUR SPECIES OF BARNACLES AND FOUR SPECIES OF TOP-SHELLS

(Figures in brackets from Evans, 1948.)

Species	Time in hours to reach 50 % mortality at temperatures (° C) of								Point of heat coma (50%) in ° C	Lethal point (50 %) in ° C	Lethal point (100 %) in ° C
	−10	−5	0	30	35	37	40	50			
Chthamalus stellatus	12–24	72–120	∞	—	—	—	29–30	½	43	52·5	53·7
Elminius modestus	12–24	48–72	∞	—	—	7½	5¼	—	36–38	48·3	49·5
Balanus perforatus	< 3	< 22	∞	—	—	—	3–5	—	38–40	45·5	47·0
B. balanoides	12–24	120–190	∞	—	—	¾	¾	—	35–37	44·3	45·3
Monodonta lineata	2–3	6–24	138–179	72–100	—	—	3 (6–6¼)	—	— (38–39)	45·0 (34·8)	45·3
Gibbula umbilicalis	2–3	16	30–79	24–72	— (10)	2	¾–1 (¾–1)	—	— (33–34)	41·8 (42·1)	42·0
G. cineraria	2–3	2–3	12–30	3¾ (5–5¼)	—	— (1¾–1½)	—	—	— (34–35)	35·5 (36·2)	36·0
Calliostoma zizyphinum	2–3	2–3	12–24	3½	—	—	—	—	33–34	34·5	34·8

TABLE 3. HEAT TOLERANCES OF SOME OTHER INTERTIDAL MOLLUSCS

(Data condensed and re-arranged from Evans, 1948.)

Species	Point of heat coma in ° C	Lethal point (50 %), ° C	Times in hours to 50 % mortality at temperatures (° C) of			Normal zonation	Distributional status (from Crisp & Southward, 1958)
			30	35	40		
Littorina neritoides	38	46·3	—	—	14–15	Supralittoral fringe and midlittoral	Southern, not in S. North Sea or eastern Channel
L. littorea	39	46·0	—	—	11½–12	Midlittoral	Intermediate, all round Britain
L. saxatilis	37	45·0	—	—	9½–10	Supralittoral fringe and midlittoral	Northern, all round Britain
L. obtusata	36	44·3	—	—	4¼–4½	Midlittoral	Intermediate, all round Britain
Patella depressa	37–38	43·3	—	—	3½–3¾	Midlittoral	Southern, S.W. England and Wales only
P. vulgata	37	42·8	—	10	2¾–3	Midlittoral	Intermediate, all round Britain
P. aspera	37	41·7	—	8½–9	¾–1	Lower midlittoral and infralittoral fringe	Southern, not in S. North Sea or eastern Channel

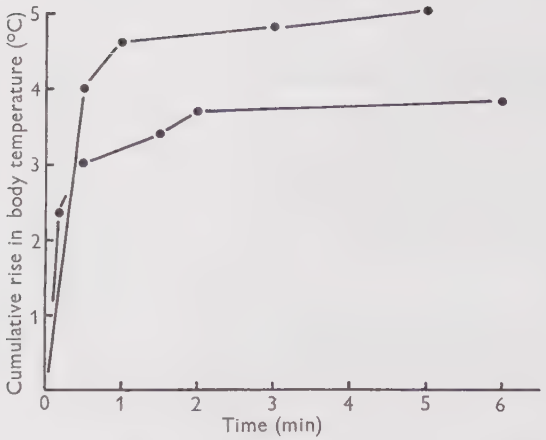

Fig. 2. Results of two experiments showing the time taken for equilibration between the tissues and the environment in a top-shell. A thermocouple was sealed inside the shell with dental cement, and the animal (15 mm diam.) transferred directly from room air temperature to sea water some 4 to 5° warmer.

Other molluscs

Time did not permit any experiments on further species of intertidal animals. However, the present results for top-shells were very little different from those recorded by Evans (1948) for the same species from Cardigan Bay (see Table 2); it seems permissible, therefore, to review Evans's results for other molluscs from the more general point of view of distribution. The heat tolerances of several littorinids and limpets from Cardigan Bay, as determined by Evans, are given in Table 3. In these animals, as in barnacles, the temperature tolerance seems connected with the geographical distribution of the species. However, the distributional differences of the vertical zonation are less marked than in barnacles and the differences in temperature tolerance seem likewise less obvious. Of the species of *Littorina*, *L. saxatilis* and *L. obtusata* are of somewhat more northern character than *L. neritoides*. This may explain the lower heat tolerance of the two former species, but it must be noted that all occur at least as far south as Portugal. Apparently, however, the littorinid most tolerant to heat, *L. neritoides*, is found the farthest south, and occurs in North Africa. No doubt the habitat of *L. obtusata*, which is normally found among fucoids, may be connected with its intolerance of high temperature.

Among the limpets, the most southerly species, *Patella depressa*, which is found only on a limited part of the south-west coasts of Britain, was found by Evans to be the most resistant to high temperatures. The least tolerance of high temperature was shown by *P. aspera*, which, though it has a more southerly distribution than *P. vulgata*, is very much a low-water species and occurs above mid-tide level only where the shore is wetted, and cooled, by considerable wave action.

Some of the above species of littorinids are found in northern Russia, where their tolerance of low temperature has been noted by Gurjanova, Sachs & Uschakov (1930). The most northern of them, *Littorina saxatilis*, was most tolerant of low temperature, and survived more than 27 h in air at −9·4°, compared with about 4 h by *L. obtusata*; *L. littorea* was killed in 3 h at −8·8°. The same workers found that *Balanus balanoides* survived 48 h at −9·4°; compared with the present results this suggests the species may possess greater tolerance in more northerly latitudes.

TEMPERATURES IN THE INTERTIDAL ZONE

The most important temperatures on the shore, from a biological point of view, are those experienced by the animals themselves. Even though the normal inhabitants of the zone are all poikilothermic, it cannot be assumed that they adopt the temperature of the sea or the temperature of the air directly they are exposed to these media. In air, when the animals are exposed to fluctuating humidity and heating up by sunlight, it cannot be assumed that their tissue temperatures ever agree with meteorological values of air temperature. With terrestrial arthropods the effects of sunlight on body temperature are remarkable (Parry, 1951; Edney, 1953).

As measurements of the animal's body temperature cannot be made very often, it is necessary to try to relate them in a general way to meteorological values of sea and air temperature and to sunlight and humidity, before any conclusions can be made regarding temperature trends in the intertidal zone. In the present work, observations were made on 13 days over a period of 10 months in 1953–4. No very hot weather occurred during this period, and it was not possible to use the apparatus in rain, but a variety of other weather conditions was observed.

METHODS

The apparatus was planned after making some preliminary measurements at Port Erin, Isle of Man. The temperatures of the animals, of tide pools and of the air close to the ground were obtained with fine-wire thermocouples. Copper/constantan couples were finally chosen because of their ease of manipulation, even though the e.m.f. provided is lower than with chromium alloys. The measuring parts of the couples were made of 10 cm of 36 B & S gauge, insulated with thinned cellulose lacquer, and were connected to the galvanometer by flexible leads, 0·5 m long, of 50 strands 36 B & S insulated with PVC sleeving. The e.m.f. was measured against a reference junction in a Thermos-flask of ice and water (temperature 0·1–0·2° by mercury thermometer), and calibrated empirically against a certificated mercury thermometer. A galvanometer of taut suspension reflecting type[1] was used with an arrangement of copper switches that enabled any of 18 couples to be compared with the reference junction or one another. Under these conditions a deflexion of up to 18 mm per degree Centigrade was achieved, and ranges of less sensitivity up to 50° f.s.d. could be obtained by use of switched shunts. An apparatus of this type was more portable and stable than valve voltmeters or a.c. bridges.

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[1] Tinsley type 5195 temperature indicator.

Humidity close to the ground was measured with Edney paper hygrometers, checked occasionally against a wet and dry bulb instrument. Sea temperatures close to the shore were taken with a mercury thermometer of the standard meteorological pattern for the purpose.

In the absence of a solar radiation meter, an approximate index of sunlight intensity was given by an incident light exposure meter[1] calibrated roughly in kilolux by reference to a photocell light recorder belonging to Dr W. R. G. Atkins, F.R.S. The meter was pointed at the sun, and recorded both sunlight and general skylight from the same direction.

Screen air temperatures for Mount Batten, Plymouth, have been extracted from *The Daily Weather Report* (Air Ministry, 1953–4) and converted to the nearest 0·5°.

All but one series of measurements were made on the rocks below the Plymouth Laboratory, at a level just below M.H.W.N. The procedure was as follows: (i) galvanometer placed on level surface and reference junction plugged into flask of ice and water; (ii) hygrometer put down; (iii) two thermocouples placed inside two barnacles through holes pierced in the shells; two more placed into limpets in the same way; two inanimate bodies, consisting of dead barnacle shells filled with plaster of Paris and each containing a couple, set up close to live barnacles but not quite touching rock surface; (iv) one thermocouple set up 1 cm from rock surface in shade of galvanometer; other couples, if available, inserted into top-shells or tide-pools; (v) sea temperature and sunlight measured while couples reached equilibrium; (vi) finally each couple switched in turn to reference junction and reading noted. In all cases several readings were taken, while with the air close to the ground, the temperature of which fluctuated continually, the maximum and minimum during 1 min were noted and the average taken.

In one series of observations at Wembury the above procedure was followed, but readings were made at several tide levels.

RESULTS

The results of the observations of temperature, humidity and sunlight on the shore at or near Plymouth are presented in Table 4, where possible to the nearest 0·1°. Some earlier observations at Port Erin, under weather conditions that were not studied at Plymouth, are given in Table 5; in this case the pointer galvanometer used allowed readings to the nearest 0·5° only.

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[1] Weston Master II, with Invercone attachment.

TABLE 4. OBSERVATIONS OF TEMPERATURES IN THE INTERTIDAL ZONE AT OR NEAR PLYMOUTH

(For further explanation refer to text.)

			Meteorology			Conditions on the shore											
Date	Time, h G.M.T.	Hours after high water	Remarks on weather	Screen air temp., noon	Sea temp. inshore	Sunlight in kilolux	Percentage relative humidity	Shade air temp. close to rocks	Temp. of inanimate body		Tissue temp. of barnacles		Tissue temp. of limpets		Temp. of tide-pools	
									Range	Average	Range	Average	Range	Average	Range	Average
22. x. 53	10	5	No sun: rocks wet	14·5	14·3	—	—	15·0	15·3–15·7	15·5	14·6–14·9	14·7	14·6–14·9	14·7	14·3–14·8	14·5
28. x. 53	16	7	Strong sun after warm day	12·0	14·0	10	—	15·0	—	16·7	—	22·4	—	17·4	—	—
28. x. 53	16	7	Hazy sun after warm day	12·0	14·0	5	—	14·2	—	15·1	—	18·2	—	17·9	—	—
4. xi. 53	10	6	Cool: a little sun at first	12·0	12·5	6	—	12·2	—	12·4	—	13·7	—	13·1	—	11·4
4. xi. 53	11	7	After an hour with full sun	12·0	12·5	35	—	13·6	17·2–20·6	18·6	—	19·6	—	18·5	—	11·7
18. xi. 53	10	7	Cool, with hazy sun	7·0	11·9	10	85	6·8	—	7·4	—	7·2	—	7·9	—	8·6
18. xi. 53	11	8	After an hour in hazy sun	7·0	11·9	15	93	7·5	9·4–9·9	9·6	8·8–9·3	9·0	—	9·6	—	—
8. xii. 53	12	6	Weak sun	12·0	12·8	10	88	13·6	13·4–13·8	13·6	13·0–13·3	13·1	—	13·2	12·3–13·2	12·8
18. xii. 53	10	7	Weak sun: thin cloud	10·0	12·2	15	93	11·4	—	12·5	—	12·4	—	12·4	—	11·4
18. xii. 53	11	8	After hour in stronger sun	10·0	12·2	30	—	12·4	—	16·2	—	15·5	—	15·5	—	12·2
2. ii. 54	11	7	Cold spell, sunny: ice on pools	2·0	6·6	25	64	—	—	—	—	—	7·8–8·2	8·0	−0·5–4·0	2·8
2. ii. 54	11	7	As above, but animals in shade	—	—	—	—	—	—	—	—	—	1·2–2·6	1·9	—	—
4. ii. 54	13	7	Cold spell: sunny	0	6·3	30	57	3·9	9·2–11·2	10·2	9·8–11·2	10·5	5·6–9·4	7·5	4·4–5·3	4·9
4. ii. 54	13	7	As above but animals in shade	—	—	—	—	—	—	—	—	—	—	4·0	—	—
1. iii. 54	10	8	Light sun	4·5	7·6	7	—	6·1	—	7·7	6·6–7·5	7·0	—	6·4	—	4·6
1. iii. 54	10	8	Becoming cloudy	4·5	7·6	7	—	5·8	—	6·4	6·0–6·7	6·3	—	5·4	—	4·9
11. iii. 54	15	5	Sunny spring day	14·0	9·4	50	45	14·3	20·6–22·2	21·4	18·5–19·4	18·9	15·4–17·2	16·2	—	10·7
17. v. 54	12	7	Sunny	14·5	12·2	75	49	17·1	22·2–23·0	22·6	24·1–24·4	24·2	—	18·0	14·8–15·0	14·9
17. v. 54	12	7	Hazy cloud passing over	14·5	12·2	—	49	16·8	21·4–21·7	21·5	20·6–21·4	21·0	—	18·4	14·8–15·0	14·9
19. v. 54	12	6	Wembury: H.W.N.; sun and breeze	16·5	13·7	75	46	20·6	26·3–28·7	27·4	27·6–28·4	28·0	24·4–25·5	24·9	16·4–17·2	16·8
19. v. 54	13	7	Wembury: L.W.N.; stronger breeze	16·5	13·7	75	58	16·8	19·6–20·6	20·2	21·4–22·5	21·9	20·1–20·4	20·2	13·6–13·9	13·7
19. v. 54	15	9	Wembury: H.W.N.; sheltered from wind	16·5	13·7	75	47	20·7	25·2–27·4	26·7	28·4–31·4	29·9	—	—	—	—
19. v. 54	15	9	As above, animals in shade	—	—	—	—	—	—	—	—	—	19·6–22·2	20·9	—	—
14. viii. 54	10	5	Sunny	16·5	14·6	75	59	17·7	25·5–27·3	27·1	23·0–23·9	23·4	—	20·9	—	17·4
14. viii. 54	11	6	An hour's further exposure	16·5	14·6	75	59	17·8	27·9–28·9	28·4	26·5–27·1	26·8	24·7–24·9	24·8	—	17·4

General inferences

Some generalizations can be made, from Tables 4 and 5, regarding temperatures in the intertidal zone. Clearly, screen air temperature, even when read at a nearby site, may have very little reference to conditions on the shore. Thus in the colder months when the sea is usually warmer than the land, and in cloudy weather when the humidity is high, the air temperature close to the rocks and the body temperature of the animals may be higher than screen air temperature even 7–8 h after being uncovered by the sea. No doubt, heat storage by the rocks may assist in maintaining temperatures. Again in the colder months, but in sunny weather, when the humidity is less, evaporative cooling on exposure to the air is counterbalanced by radiant heating from the sun, and the air temperature close to the rocks and the body temperature of the animals are usually much higher than either screen air temperature or sea temperature. No doubt at night in cold clear weather, evaporative cooling may produce temperatures below that of the sea; but although measurements have not been possible in the dark, it has been noticed that the rocks remain quite wet, hence evaporative cooling cannot be very marked.

TABLE 5. OBSERVATIONS OF TEMPERATURE IN THE INTERTIDAL ZONE AT OR NEAR PORT ERIN

(For further explanation refer to text.)

			Meteorology				Conditions on shore				
								Tissue temp. of barnacles		Tissue temp. of limpets	
Date	Time, h G.M.T.	Hours after high water	Remarks on weather	Screen air temp.	Sea temp. inshore	Percentage relative humidity	Shade air temp. close to rocks	Range	Mean	Range	Mean
23. ii. 50	10	7	Cloudy	—	8·0	87	9·0	8·0–8·5	8·0	8·0–8·5	8·0
22. xi. 50	16	6	Low cloud, rain at times	6·5	9·5	85	7·0	—	7·0	—	7·0
13. xii. 50	10	8	Cold spell, cloudy	0	9·0	67	1·5	—	2·0	—	3·5

In the warmer months of the year, and in sunny weather, temperatures on the shore after the tide has fallen are well above sea temperature and usually higher than screen air temperature. Again at night evaporative cooling may take place, but again the shore has been observed to remain wet. In cloudy weather in that part of the year when air temperatures exceed sea temperature during the day there may be closer agreement between screen air temperature, the air temperature close to the rocks and the temperatures of the animals themselves, but even the slightest amount of sunlight filtering through the clouds causes warming up of the shore.

To sum up, it seems that, in daylight, intertidal animals exposed to the air will often have higher body temperatures than would be expected from sea and air temperatures. This is clearly brought out if we compare the maximum, minimum and mean temperatures of the observation period (Table 6). The minimum temperatures of the animals may be below the minimum sea temperature, but both means and maxima are well above the corresponding values of sea and air. Compared with the temperature of the tide pools or the air on the shore, the animals show an upward shift of the range of temperatures experienced. Some of this shift may be due to retention of the warmth of the sea, but most of it can be attributed to the warming effects of sunlight. These effects are not felt by animals that are always in the shade, and such animals, as far as can be ascertained from the few observations made, are subject to a lower annual range of temperature. Animals in the shade may follow shade air temperatures more closely than those on the open rock, but should never show temperatures as low as the extreme screen temperatures.

TABLE 6. ANALYSIS OF OBSERVATIONS SHOWN IN TABLE 4

(For the air on shore, the limpets, the inanimate body and the barnacles the figures refer to the average of the readings on each occasion. Figures for animals in the shade are shown in parentheses.)

	Temperature		
Medium or animal	Maximum	Mean	Minimum
Screen air	16·5	10·1	−2·0
Tide pools	17·4	10·8	2·8
Sea inshore	14·6	11·3	6·3
Air on shore	20·7	13·3	3·9
Limpets	24·9 (20·9)	14·2	5·4 (1·9)
Dry, inanimate body	28·4	17·0	6·4
Barnacles	29·9	17·3	6·3

Effect of sunlight and wind

It has already been remarked that an upward shift in the range of temperatures experienced by animals on the open rock can be attributed in large part to warming by sunlight. Comparison of the temperatures of the living animals and of the inanimate bodies not in contact with the rock (the dead barnacle shells) shows how much of this warming up is direct or by conduction from the layer of warm air close to the ground (cf. Parry, 1951), and not by conduction from the heated rocks (Fig. 3). Only on days when there had been prolonged heating by the sun, and the rocks were thoroughly warmed up, were the living animals at a higher temperature than the inanimate bodies. With limpets, however, there was somewhat less agreement between their tissue temperatures and those of inanimate bodies above 20°. No doubt this difference reflects the larger size of the limpets and their correspondingly lower proportion of surface area.

The effect of sunlight is well illustrated by the records for 4 November and 18 December in Table 4. On the latter occasion an hour's exposure to a twofold increase in sunlight raised the shade temperature of the air close to the rocks by 1° only, while the animals rose 3–4°; on the earlier date the sunlight increased nearly sixfold, and the animals rose by 5° or 6° compared with an increase of only just over 1° in shade air temperature. Such heating up by sunlight can be very rapid. On one occasion after a light cloud had passed from in front of the sun a barnacle increased by 1° in 1 min. On another occasion on the laboratory roof a top-shell practically doubled its temperature, from 17·6° to 31·4°, in 35 min. The results of the latter experiment are shown in Table 7, with readings of sunlight and the temperatures of an inanimate body (one of the dead barnacles filled with plaster of Paris).

In the above experiment there was little or no movement of the air. Parry (1951) has shown the importance of the layer of warm air close to the ground in the heating up of small bodies in sunlight. Clearly, the presence of wind will modify the heating-up process, and should permit cooling by convection. This is borne out by the data from Wembury on 19 May (Table 3). The difference between an absolute calm and a light breeze under otherwise similar conditions amounted to some 1–3° in body temperature of the barnacles.

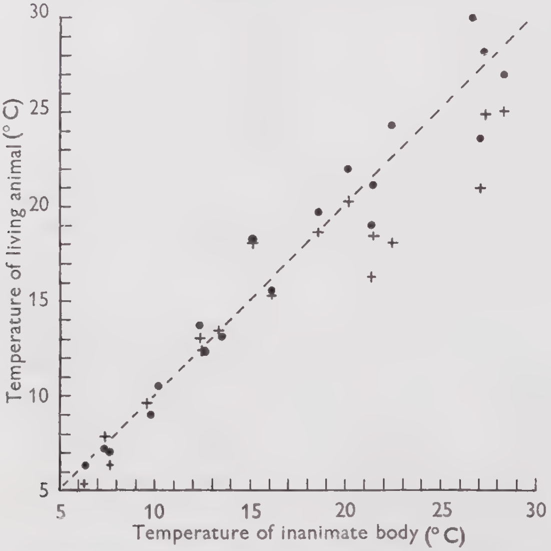

Fig. 3. The body temperatures of barnacles (dots) and limpets (crosses) in sunlight, plotted against the temperatures assumed by an inanimate body. The dotted line represents perfect agreement between the animate and inanimate bodies. For further details refer to text.

TABLE 7. TEMPERATURES OF A LIVING TOP-SHELL AND AN INANIMATE BODY* IN SUNLIGHT AT PLYMOUTH

(On roof of laboratory, no wind: taken directly from room temperature.)

	Illumination from sun and part of sky in approx. kilolux	Top-shell (° C)	Inanimate body (° C)
At start	30	17·6	16·3
10 min later	35	20·4	18·9
13 min later	40	23·9	20·8
30 min later	60	31·3	29·3
35 min later	60	31·4	30·0

* A dead barnacle shell filled with plaster of Paris.

DISCUSSION

The experiments and field measurements that have been described above were carried out in support of work on distribution. For this reason they were not continued when it became clear that actual temperatures experienced on the shore were, in most cases, well inside the tolerance limits of the animals. This might have been expected, for all the animals live successfully at thc places where the temperatures were measured.

It must be remembered that more extreme conditions occasionally experienced, even if only for a short period, might have lethal effects. This is particularly so with the northern species, such as *Balanus balanoides*, which is near the limits of its tolerance of high temperature. On one occasion in the Isle of Man temperatures up to 38° were measured in barnacles (*Chthamalus*) above M.H.W.N., while Moore (1935) records a temperature of 36·5° in a barnacle at Plymouth. Temperatures of this order can produce coma in *Balanus balanoides*, and cause over 50 % mortality if experienced for more than 45 min. Thus the detrimental effects of heating up in the sun may contribute to the geographical limit of *B. balanoides*, and may help to restrict it to low tide levels or shaded habitats near its southern boundary. The remaining species, however, appear capable of withstanding even the exceptional conditions, and there is no evidence for limitation of their habitat.

There is little possibility of directly lethal effects of occasional low-temperature extremes, even if we consider temperature conditions at a locality where the less tolerant species are absent. For example, from Table 2 it can be seen that exposure of the midlittoral species *B. perforatus*, *Monodonta lineata* and *Gibbula umbilicalis* to temperatures lower than $-5°$ might cause heavy mortality within one tidal period (6–12 h). During the course of the work there was a fairly long cold spell with consistently subzero temperatures; Table 8 shows the values of screen air temperature at 6-hourly intervals for 10 days of this cold spell at Plymouth and at a coastal station in the eastern Channel, where these animals are absent. Only on one occasion at Plymouth did temperatures fall below $-5°$, and although this low temperature was maintained for more than 6 h it coincided with a rising tide and the intertidal zone was exposed for only a few hours. At the other station temperatures fell below $-5°$ on five occasions, several times for more than 6 h and once for over 18 h (nearly two tidal cycles). The significance of this difference between the two stations is reduced if we consider the evidence of the field measurements of the animals' temperature. The species in question have body masses more comparable to limpets than to the barnacles (*Chthamalus*) used in the field work; nevertheless, even limpets in the shade had temperatures well above zero at Plymouth on the coldest day of the cold spell, while animals in the sun were as much as 8–10° warmer than the screen air at midday. There is thus little likelihood that lethal temperatures were experienced on the shore at Plymouth during this cold spell, though the presence of ice on the tide pools shows that temperatures on the shore were sometimes below −2°. If the temperature differentials between the animals and the screen air temperature can be applied also to Lympne, the more easterly station, there is little possibility that directly lethal temperatures were experienced there either, even if allowance is made for the much lower values of air temperature recorded.

It is clear that during a cold spell the rocks and the animals on the shore retain the warmth of the sea while out of water long enough to maintain temperatures above zero during the period of exposure, while the smallest amount of sunshine should immediately warm them up. The only circumstances that can be envisaged as having directly lethal effects are those existing during the exceptional cold spells that occur in certain years, when the sea temperatures inshore may be below zero and there is little sunshine (see Orton, 1931). Such circumstances are not experienced very often, and are hardly regular environmental factors, even in eastern England; moreover, they can kill other animals apart from any strictly southern species.

TABLE 8. COMPARISON OF SCREEN AIR TEMPERATURES AT PLYMOUTH AND LYMPNE

(Readings at 6-hourly intervals during a cold spell in 1954. From Air Ministry, 1954. Converted from ° F to nearest 0·5° C.)

	Lympne					Plymouth				
	Temperature at hours:					Temperature at hours:				
Date	0	6	12	18	Hours sun	0	6	12	18	Hours sun
28. i.	−5·5	−8·0	−4·0	−4·5	4·5	0·5	0	0	−1·0	0
29. i.	−2·0	−0·5	−1·5	−0·5	2·2	−3·0	−1·0	0·5	1·0	0·3
30. i.	0	−3·5	−1·0	−4·0	2·2	0·5	1·0	0	−0·5	7·2
31. i.	−6·0	−5·0	−6·0	−5·0	5·0	−1·5	−1·5	−1·0	−4·0	6·6
1. ii.	−4·5	−6·5	−4·5	−5·0	6·8	−4·0	−4·0	−1·5	−3·0	7·3
2. ii.	−5·0	−4·5	−3·0	−3·5	1·9	−5·5	−5·5	−2·0	−1·0	7·0
3. ii.	−3·5	−4·0	−1·5	−1·5	8·3	−3·5	−2·0	0·5	−1·0	6·9
4. ii.	−1·0	−3·0	−1·5	−3·0	6·6	−3·0	−1·5	0	−1·5	5·2
5. ii.	−4·5	−4·5	−1·5	−3·0	5·7	−3·0	−2·0	−2·0	−2·0	1·5
6. ii.	−5·0	−7·0	−3·0	−1·5	0·5	−2·0	−3·5	0	1·0	3·3
	Mean −3·56					Mean −1·55				

In a more severe climate than that of Western Europe it is possible that direct lethal effects of low temperature may be a normal occurrence and may influence the distribution of the less hardy forms (see Kanwisher, 1955). In Western Europe, at any rate, evidence for a causal relationship between temperature and distribution must be sought in non-lethal terms such as debilitating effects at some stage in the life history. Dr D. J. Crisp informs me that some preliminary experiments he has made show that among the barnacles the southern forms may take longer to recover from chill coma than the northern species, and may thus lay themselves open to selective attack by predators. Other effects of temperature may be found in combination with other factors or indirectly through competition between species (Southward & Crisp, 1956; Crisp and Southward, 1958).

This work was carried out during the tenure of a D.S.I.R. Senior Research Award. Prof. J. F. Teago, Dr L. Jacob and Dr D. A. Parry kindly advised me on apparatus, which was purchased with a grant from the Royal Society. I am indebted to the Micanite and Insulators Co. for samples of insulating sleeving and to H. Tinsley and Co. for their readiness to modify their galvanometers to my needs. The manuscript has been criticized by Dr D. J. Crisp to whom I am grateful for many useful suggestions.

SUMMARY

In experiments on four species of barnacles and four species of top-shells, the barnacles were found to be more resistant to high or low temperatures than the top-shells. Among each group of animals the degree of tolerance was related to the geographical distribution of the species and their zonation on the shore. Thus most tolerance of high temperature was shown by species of southern distribution, especially by those occurring at the upper limit of the midlittoral zone, and most tolerance of low temperature by species of northern distribution. Least tolerance of high and low temperatures was shown by species found only in the infralittoral fringe or below low water.

Field measurements of the body temperatures of barnacles and limpets while exposed to the air were made with thermocouples. Under many weather conditions the body temperatures were higher than would be expected from local meteorological values of air temperature. This difference was due to retention of sea temperature by the animals and the rocks and to the heating effects of sunlight.

The results of the laboratory experiments and of the field measurements are discussed together in relation to weather and geographical distribution. It is clear that the temperatures experienced on the shore are well within the tolerance limits of most of the animals, and even exceptional extremes of temperature may have little direct influence on the distribution of adult intertidal animals. Evidence for a causal relation between temperature and distribution must be sought in non-lethal terms such as debilitating effects, or indirectly through competition between species, or in combination with other factors.

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THE ADHESIVE MECHANISMS OF MONOGENETIC TREMATODES: THE ATTACHMENT OF SPECIES OF THE DICLIDOPHORIDAE TO THE GILLS OF GADOID FISHES

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(Plate I and Text-figs. 1–3)

An account of the adhesive organs of *Diclidophora denticulata* and of the attachment of this parasite to the gills of *Gadus virens* was given by Cerfontaine (1896, 1898) in what has come to be regarded (Dawes, 1947) as a classic piece of description. The adhesive organs of other species of *Diclidophora* have been described by Gallien (1937) and Brinkmann (1942), while Price (1943), Sproston (1945, 1946), Dawes (1946, 1947), Chauhan (1953), and Hargis (1955) have made extensive use of the structure of adhesive organs in systematic surveys of the Diclidophoroidea. However, a consideration of all these accounts, in order to compare the structure of the adhesive organs of the Diclidophoridae with those of *Plectanocotyle gurnardi* and *Kuhnia scombri* which I have described previously (Llewellyn, 1956*a*, 1957*a*), showed that some of the more recent accounts were in very considerable conflict with Cerfontaine's description of *Diclidophora denticulata*, but that no actual comparisons had been made. In the present paper a comparative study is made, from actual specimens, of what are probably all of the valid species reported so far as belonging to the genus *Diclidophora*.

Specimens were obtained from the sources indicated in Table 1, and have been named as in Sproston (1946, pp. 469–85). In addition to the species of *Diclidophora* listed by Sproston, *D. gadi* (Reichenbach-Klinke, 1951), syn. *Dactylocotyle gadi* Reichenbach-Klinke, 1951, has been studied from whole-mount preparations. It has not been possible for me to examine *Diclidophora maccallumi* (Price, 1943) Sproston, 1946, but from Price's diagram (1943, fig. 5) of the adhesive organ, this species seems more likely to belong to the Cyclocotylinae than to the Diclidophorinae. With regard to *Diclidophora morrhuae* (van Beneden & Hesse, 1863) Sproston, 1946, it seems probable that no such animal exists: Dawes (1947, p. 107) stated that the identity of the species was very doubtful; Gallien (1937) searched for, but did not find any monogenean on cod; and I have examined about fifty specimens of *Gadus morrhua* without finding a trematode gill parasite; a comparatively great amount of fisheries work has been done on cod, and it is unlikely that a gill trematode would have escaped attention.

The histological techniques used have been those described previously (Llewellyn, 1956*a*, 1957*a*).

TABLE 1. SOURCES OF *DICLIDOPHORA* MATERIAL

Parasite	Host	Locality	Collector
D. merlangi	*Gadus merlangus*	Aberystwyth Plymouth	Rees & Llewellyn (1941) Llewellyn (1956*b*)
D. denticulata	*G. virens*	Irish Atlantic Slope St Andrews	Rees & Llewellyn (1941) Frankland (1955)
D. luscae	*G. luscus*	Plymouth	Llewellyn (1956*b*)
D. macruri	*Macruris rupestris*	Skager-Rak	Brinkmann (1942)
D. minor	*Gadus poutassou*	Skager-Rak and North Sea	Brinkmann (1942)
D. palmata	*Molva molva*	Irish Atlantic Slope Iceland (64° 20′ N., 14° 19′ W.)	Rees & Llewellyn (1941) King Edward's School Five Ways Birmingham Marine Expedition, 1956
D. phycidis	*Urophycis blennoides*	Irish Atlantic Slope	Rees & Llewellyn (1941)
D. pollachii	*Gadus pollachius*	Plymouth	Llewellyn (1956*b*)
D. gadi	*G. aeglefinus*	North Sea	Reichenbach-Klinke (1951)

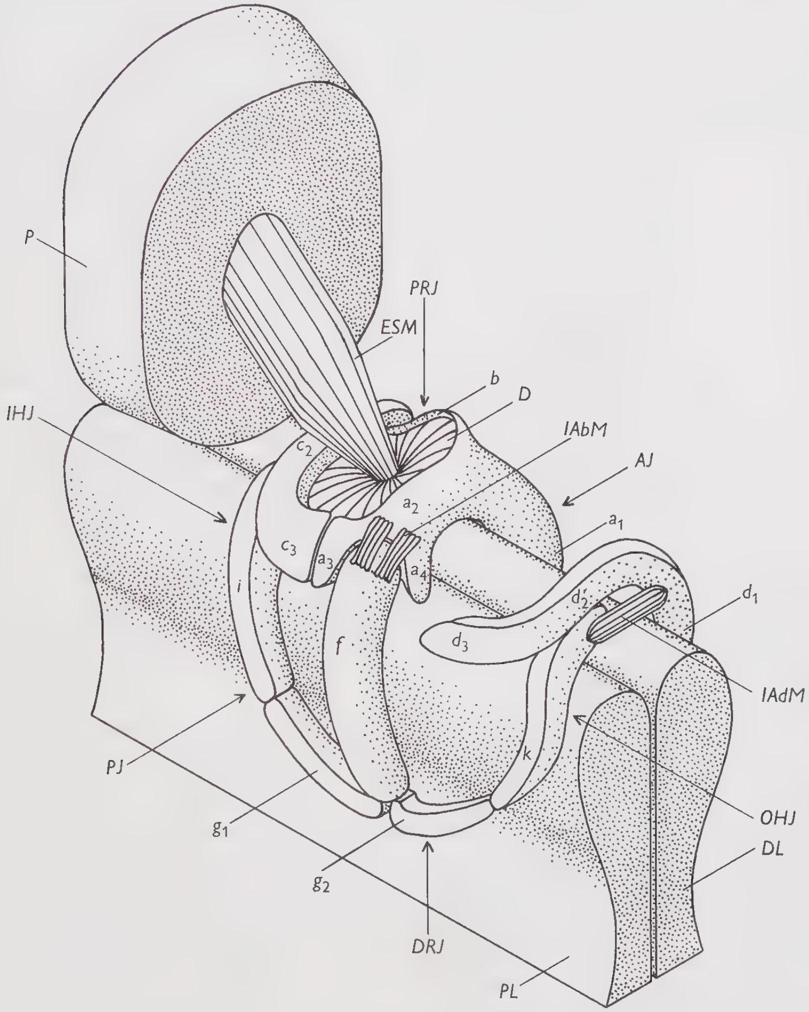
THE ADHESIVE MECHANISM OF *DICLIDOPHORA*

A comparative account of the adhesive attitudes of several species of *Diclidophora* has been given previously (Llewellyn, 1956*b*), and is further illustrated here for *D. denticulata* in Pl. I, fig. 1. In all species, each of the eight adhesive organs grasps secondary gill lamellae in exactly the same manner, and, moreover, the general structure of all of the adhesive organs in all species is very similar, and is illustrated in Text-figs. 1 and 2. There are minor specific differences, and these will be described later (pp. 72–73). As far as possible, Cerfontaine's notation for the identification of various structures has been adopted, but some modifications and extensions to his scheme have been found necessary.

The structure of the adhesive organs

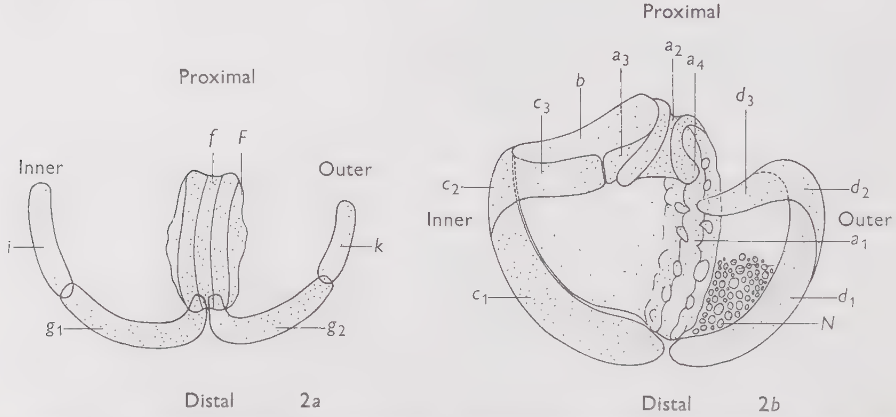
Each adhesive organ consists of a pair of dissimilar opposable hinged jaws that grasps two or three secondary gill lamellae so that the anterior jaw lies distally with respect to the origin of the primary lamella from the gill arch of the host fish, and the posterior jaw proximally (Pl. I, fig. 1). The anterior jaw is deeply hollowed and is relatively fixed, while the posterior jaw is shallow and is movable about its hinged attachment to the anterior jaw, the movement being effected by both intrinsic and extrinsic muscles. The walls of the adhesive organ are supported by sclerites (= skeletal bars, plates, nodules and teeth), the arrangement of which is asymmetrical, making it possible to recognize an 'inner' (more median) and an 'outer' (more lateral) half to each organ (Text-figs. 1 and 2).

The inner half of the anterior jaw is supported by two fused sclerites, one, a ($=a_1a_2a_3a_4$), in the median region of the jaw, and the other, c ($=c_1c_2c_3$), a peripheral bar. Bar a is a curved rod passing (a_1) from a median distal



Text-fig. 1. Stereogram of an adhesive organ of *Diclidophora* grasping secondary gill lamellae of host. a_1, a_2, a_3, a_4, b, c_2, c_3, d_1, d_2, d_3, f, g_1, g_2, i, k, sclerites (see text, pp. 69–71); *AJ*, anterior jaw; *D*, diaphragm; *DL*, distal lamella; *DRJ*, distal region of jaw; *ESM*, extrinsic suctorial muscle; *IAbM*, intrinsic abductor muscle; *IAdM*, intrinsic adductor muscle; *IHJ*, inner half of jaw; *OHJ*, outer half of jaw; *P*, peduncle; *PJ*, posterior jaw; *PL*, proximal lamella; *PRJ*, proximal region of jaw.

position in the anterior jaw to a median proximal position, where it curves posteriorly (a_2) and articulates with the corresponding median bar f of the posterior jaw. For most of its length sclerite a is hollow, but, especially in its proximal region a_2, it may be U- or V-shaped in section, with the opening of the U directed away from the cavity of the adhesive organ, i.e. towards the peduncle bearing the adhesive organ. The anterior surface(s) of the anterior part a_1 of the median sclerite a often bears irregular nodules or spines. At the proximal, posterior end of a_2, the arms of the U are produced so that the end of the sclerite is Y- (or T-) shaped, with the outer arm (a_4) of the Y terminating blindly, but with the inner arm (a_3) forming a symphysis with the proximal end of the peripheral bar (c_3) of the anterior jaw.



Text-fig. 2. The arrangement of the sclerites of the adhesive organ of *Diclidophora*. (*a*) Isolated posterior jaw, seen posteriorly. (*b*) Isolated anterior jaw, seen posteriorly. a_1, a_2, a_3, a_4, b, c_1, c_2, c_3, d_1, d_2, d_3, f, g_1, g_2, i, k, sclerites (see text, pp. 69–71); *F*, thin flange from *f*; *N*, nodules.

From the inner surface of a_1 arises a lamellate extension b which along the greater part of its marginal regions is fused to the anterior region c_1 of the peripheral sclerite c. Thus, between them, sclerites a and b, together with c, form a scoop-like structure with thickened margins and which, proximally, forms a ring bounded by a_2, the proximal border of b, c_2, c_3 and a_3. This ring suspends a diaphragm which is itself an important component of the adhesive mechanism. An extrinsic muscle which operates the diaphragm approaches it from the peduncle at an angle of about 45° to the hinge axis of the adhesive organ, and the proximal border of b is correspondingly inclined to accommodate such an arrangement (Text-fig. 2*b*).

The outer half of the anterior jaw is supported by a single solid peripheral sclerite d ($=d_1d_2d_3$) which curves in three different planes: first it borders the outer margin of the anterior jaw (d_1), secondly it curves into the proximal region of the adhesive organ (d_2), and thirdly it curves again, obliquely, into the plane of the posterior jaw (d_3) and ends blindly at some distance distal to the outer spur a_4 from a, i.e. there is no fusion between d and a.

On the anterior surface of the outer half of the anterior jaw (i.e. on the outside of the adhesive organ), in the region bounded by a_1 and d_1, there is present, in some species, a number of small sclerites, e.g. the 'teeth' of *Diclidophora denticulata* and the 'nodules' of *D. luscae*, but some species, e.g. *D. palmata*, are without corresponding structures.

The posterior jaw is supported by a median hollow rod f hinged to sclerite a of the anterior jaw, and by two pairs of solid peripheral sclerites, one pair i and k proximally with i in the inner half of the jaw and k in the outer, and one pair g_1 and g_2 distally with g_1 in the inner half of the jaw, and g_2 in the outer. The proximal ends of i and k are hinged on to the proximal arched portions c_2 and d_2 respectively of the peripheral sclerites of the anterior jaw, while the distal ends of sclerites g_1 and g_2 are immovably jointed to the distal end of the median sclerite f of the posterior jaw. In most species i and g_1 (and k and g_2) are contiguous, but in *D. denticulata* i is separated from g_1 (and k from g_2) by a considerable gap.

The inner walls of the clamp are lined with slender rib-like sclerites similar to those illustrated previously for *Plectanocotyle* (Llewellyn, 1956*a*, Pl. I, figs. 5, 6, 8).

The mode of action of the adhesive organs

Each posterior adhesive organ of *Diclidophora* is operated by two distinct but co-ordinated mechanisms. First there is a set of intrinsic muscles consisting of three adductors connecting i, f, and k of the posterior jaw with c_2, a_2 and d_2 respectively of the anterior jaw, and a median abductor, itself composed of two distinct muscle bundles, connecting f with a_2 (Text-fig. 1; Pl. I, figs. 3, 6). By use of these antagonistic intrinsic muscles the jaws may be opened and closed, and in living specimens of *D. merlangi*, forcibly detached from the host tissue, I have seen the peduncle repeatedly extended with the jaws opening, and then the jaws rapidly snapped together and the peduncle withdrawn.

The second component of the adhesive mechanism consists of a suctorial device comprising the hinged jaws with their enclosed cavity bordered by soft lips, and a fibrous diaphragm suspended in the ring formed by the proximal regions of sclerites $a_2bc_2c_3a_3$, and actuated by the powerful extrinsic muscle. The extrinsic muscles of the adhesive organs are arranged in the same manner as I have illustrated previously for *Plectanocotyle gurnardi* (Llewellyn, 1956*a*; Text-fig. 2). Other relatively small extrinsic muscles are associated with the adhesive organs, but these serve for orientating the organs, and play no part in the actual adhesive action.

The diaphragm consists of part of the fibrous wall of the adhesive organ, normally a structure consisting of fibres arranged perpendicularly between inner and outer basement membranes, in which the extrinsic muscle has perforated the outer or proximal (with respect to the peduncle) basement membrane to become inserted on to the central region of the inner basement membrane (Pl. I, fig. 3). At this region of insertion there may be sclerotization, the hardened tissue taking the form of irregular small nodules, or of a single disc, or of a ring or a pair of semicircular arcs. The degree of sclerotization of the central region of the diaphragm varies, not only among the different species of *Diclidophora*, but in different individuals and even between different adhesive organs of the same individual. Cerfontaine stated that there were such sclerotizations in all individuals of all the five species of *Diclidophora* that he had examined, and while this may well be so, it is perhaps worth while pointing out that sometimes it is only in sections that they may be identified.

The mode of action of the adhesive organs is the same in all species of *Diclidophora*. First the jaws are opened by the abductor muscle, then they are applied to two or three secondary gill lamellae, and a preliminary grasp is taken by the closing of the jaws by the intrinsic adductor muscles. Next follows the second and more important adhesive action: the powerful extrinsic muscle contracts, the central part of the diaphragm is withdrawn into the peduncle, the marginal lips of the jaws act as valves to seal off the internal cavity of the adhesive organ, and a suction pressure is set up in the cavity of the adhesive organ. The two jaws being freely hinged (the abductor muscle being relaxed), this pressure is converted into a clamping action which is proportional to the pull of the extrinsic muscle. The change in internal volume occasioned by the withdrawal of the diaphragm would be small, but the sea water in the clamp cavity would act as the agent for the very efficient hydraulic transmission of a suctorial action into a clamping action.

Variations in the structure of the adhesive organs in the different species of Diclidophora

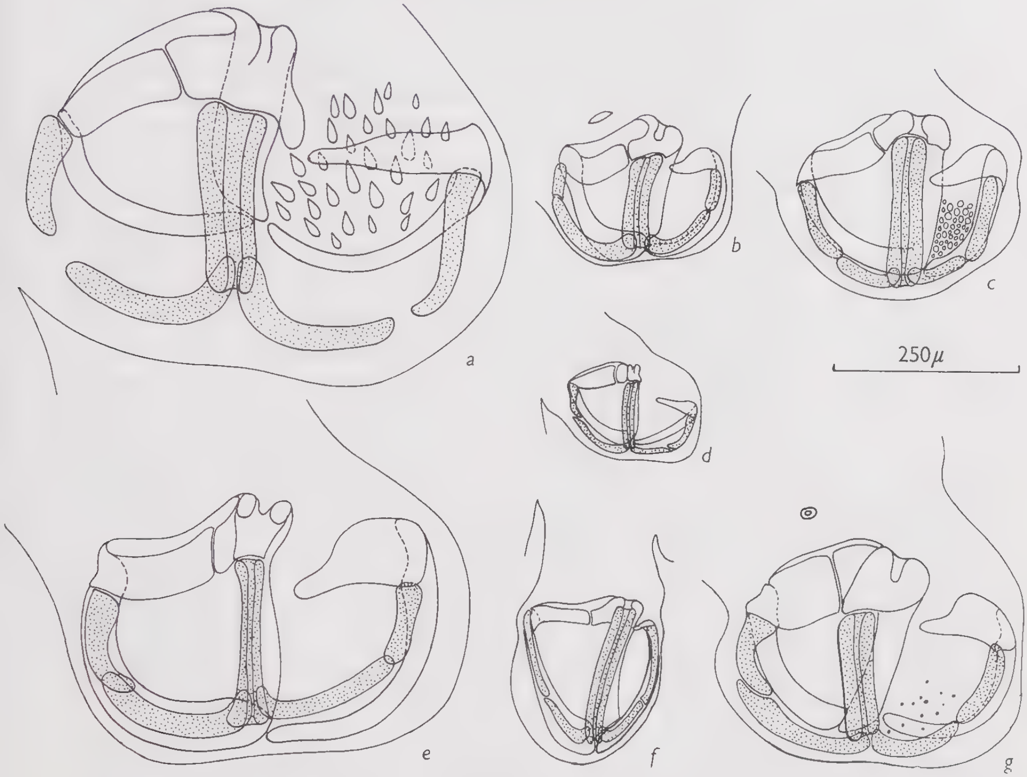
Variations in the sizes and shapes of whole organs and of their component parts are illustrated in Text-fig. 3.

D. merlangi (Text-fig. 3*g*). About 5–15 minute tubercles are present on the distal region of the outer surface of the outer half of the anterior jaw. The diaphragm sclerotization consists of a ring or of a pair of semicircular arcs.

D. denticulata (Text-fig. 3*a*). About 25–35 conical teeth are present on the outer surface of the outer half of the anterior jaw. The proximal and distal peripheral sclerites of the posterior jaw are separated from each other by a considerable gap. The diaphragm sclerotization is feeble or absent.

D. luscae (Text-fig. 3*c*). About 50–100 well-developed tubercles are present on the outer surface of the outer half of the anterior jaw. The diaphragm sclerotization is feeble or absent.

D. macruri (Text-fig. 3*f*). The width of the adhesive organ (= length of the hinge axis) is less than the length of the median sclerite *f* of the posterior jaw, i.e. the clamp is narrower than its own length, whereas the clamps of all other species are wider than their own length. This difference is accompanied by a reduction of the posterior proximal region d_3 of the outer peripheral sclerite of the anterior jaw, and a corresponding increase in the length of c_3 in the inner half of the jaw, i.e. a greater proportion of the proximal surface of each clamp is involved in the suctorial mechanism.



Text-fig. 3. Variations in size and shape of the sclerites of the adhesive organs of various species of *Diclidophora*, all drawn to the same scale. (*a*) *D. denticulata*, (*b*) *D. phycidis*, (*c*) *D. luscae* and *D. pollachii*, (*d*) *D. minor*, (*e*) *D. palmata*, (*f*) *D. macruri*, (*g*) *D. merlangi* and *D. gadi*.

D. minor (Text-fig. 3*d*). The sclerotization of the diaphragm is feeble or absent. No special features are present.

D. palmata (Text-fig. 3*e*). The sclerotization of the diaphragm is feeble or absent. No special features are present.

D. phycidis (Text-fig. 3*b*). The sclerotization of the diaphragm consists of a single well-developed disc.

D. pollachii (Text-fig. 3*c*). The adhesive organ resembles exactly that of *D. luscae*.

D. gadi (Text-fig. 3*g*). The adhesive organ resembles exactly that of *D. merlangi.*

Comparison with previous accounts of the adhesive organs of Diclidophora

Cerfontaine (1896, 1898) gave a generally accurate account of the adhesive mechanism of *Diclidophora*, but was mistaken in some aspects of the structure of the adhesive organs. The posterior jaw was thought to be supported by seven sclerites, but in fact only five of those described by Cerfontaine belong to this jaw, and the other two are parts of sclerites of the anterior jaw. In particular, Cerfontaine's discrete sclerite '*h*' of the posterior jaw is really a composite structure $c_3a_3a_4$ belonging to the anterior jaw, and similarly Cerfontaine's '*l*' of the posterior jaw is really d_3 of the anterior jaw. Cerfontaine regarded '*c*' ($=c_1$) as being the thickened margin of *b*, but in fact c_1 is a separate sclerite that is joined only in part to *b*. It is questionable whether Cerfontaine's hypothesis of a 90° rotation of each clamp to bring the 'ventral' valve to an anterior position and the 'dorsal' valve to a posterior position in order to bring about alignment with the secondary gill lamellae is really necessary: as may be seen by comparing *Diclidophora* with *Plectanocotyle* and *Kuhnia* (Llewellyn, 1956*a* and 1957*a* respectively), the anterior and posterior jaws probably occupy their normal positions.

Both Gallien (1937) in describing *Diclidophora minor* and Brinkmann (1942) in describing *D. macruri* in general repeated Cerfontaine's error in regarding the system c_3a_3 (= Brinkmann's 'B6') as a single discrete sclerite.

Price (1943) diagnosed the family Diclidophoridae with reference to a diagram of the clamp structure. No further description was given, and the clamp illustrated was almost certainly one that had been forced open beyond its normal limits, and which was seen in distal view. Price's diagram is reproduced here (Pl. I, fig. 2*a*) alongside a photograph of a similarly treated clamp (Pl. I, fig. 2*b*) in order that corresponding parts may be identified.

Dawes (1946, p. 47) gave a diagnosis of the Diclidophoridae with reference to Price's (1943) unlabelled illustration of the sclerites, which was reproduced, and qualified the diagram with a description of the arrangement of the sclerites. Later, Dawes (1947) repeated this description, and gave a translation of Cerfontaine's account of the adhesive organs of *D. denticulata* accompanied by a reproduction of Cerfontaine's diagrams, and also included original diagrams, but without descriptions, of the adhesive organs of *D. merlangi*, *D. palmata* and *D. denticulata*. According to Dawes's description of Price's diagram, there are eight sclerites in all:

(i) 'Four pieces bordering the opening of the sucker ventrally.'

According to my interpretation, Price's diagram shows two or at most three such sclerites, and in fact there are two (c_1 and d_1).

(ii) 'Two others bent at right angles, one part of each bordering the opening dorsally, the other being directed towards its centre.'

These I take to be g_1 and g_2 bordering the opening, with f directed towards the centre; such interpretation would impute a double structure to f when it is in fact a single hollow bar (as was illustrated in Dawes, 1947, fig. 18*c*, in a reproduction of Cerfontaine's figure) that appears double as seen in optical section as shown in Pl. I, fig. 2*b*, and would make no provision for i and k, which are shown clearly in Price's diagram.

(iii) 'Two others reinforcing the dorsal wall, one T-shaped, the other continuing the stem of the T above its limbs (thus †).'

In the 1947 publication (p. 94) the description of these last two sclerites (i.e. (iii) above) was altered somewhat, but still referred to them as occurring in the dorsal wall and forming a cruciform arrangement, with the stem of the T-piece having a lamellar extension and with its fellow supporting the concavity of the organ.

It seems to me that Dawes's 'T-shaped piece with lamellar extension' can only be the bar extending from the centre of Price's diagram directly to the bottom and then curving to the left of the figure, and equivalent to a_1a_2. However, both in Price's illustration and in fact sclerite a_1a_2 is in the 'ventral' (= anterior) valve. This interpretation presents a further difficulty in that the continuation of the stem above the limbs of the T would be equivalent to f, which, in my interpretation of Dawes's description, is already occupied by the right-angled centrally directed bends from g_1 and g_2.

It may be concluded then that there are some rather fundamental inconsistencies in Dawes's descriptions of the adhesive organs of *Diclidophora*.

In two accounts, that in certain respects were conflicting, Sproston (1945, p. 193; 1946, pp. 471 and 472) described the structure of the clamps of the Diclidophoridae and of the Diclidophorinae (which contains the single genus *Diclidophora*) in terms of what were thought to be the homologies of the various sclerites throughout the superfamily Diclidophoroidea. According to this scheme, the most generalized pattern of sclerites survived in the Mazocraeidae, and the diclidophorid pattern was relatively advanced. It has been shown elsewhere (Llewellyn, 1957*a*) that Sproston's interpretation of the clamp of the Mazocraeidae was inaccurate, and the present study shows that the descriptions of the clamp of *Diclidophora* are also erroneous. Among the chief mistakes are the following:

(i) The lateral walls of the single hollow median sclerite f of the posterior jaw were seen in optical section as two separate parallel bars that were then assumed to be derived from elongations of the 'middle loop' (= peripheral sclerites g_1 and g_2) so that the 'cuticularized tendon' or 'spring' (= median sclerites f and a_1a_2 in my description) were correspondingly reduced and were represented by an irregular ring in the middle of the 'anterior' (= proximal) edge of the capsule. In fact, f and a_1a_2 are the largest components of the sclerite system of *Diclidophora*, and far from being a 'spring', are articulated with each other in a hinge joint provided with adductor and abductor muscles as had been described by Cerfontaine, and as are illustrated here in Pl. I, figs. 2*b*, 6.

(ii) No reference was made to the lamellate extension *b* from sclerite *a*.

(iii) More than half the main muscles were thought to be attached directly on to the inner moiety of the 'dorsal loop' ($=c_3a_3$). In fact the whole of the main muscle (= the extrinsic muscle) is attached to the centre of the diaphragm, which is itself in part suspended by c_3a_3.

Sproston thought the adhesive organs of the Diclidophoroidea (excepting the Choricotylinae and Hexostomatidae) functioned as clamps (pincers) in which certain curved portions of the clamp skeleton were under strain when the main retractor muscles contracted, and straightened out and opened the mouth of the clamp when these muscles relaxed. (A similar arrangement had been described by Goto (1895, p. 148) in a general account of the adhesive organs of various polyopisthocotylineans, but in this case to explain a suctorial and not a clamping action). The main muscle bundle in the Diclidophoridae was described by Sproston as being attached to the 'inner remnant of the dorsal loop', and though an incipient sucker was thought to be present in the Diclidophorinae, the whole organ was thought to maintain the usual function of a clamp. Since the 'spring' ($=af$) was thought to be reduced, and the 'sucker' to be only incipient, and since no explanation was offered as to how a muscle attached to the 'inner remnant of the dorsal loop' could operate the clamp, Sproston's accounts are without any feasible explanation of a clamping mechanism. In fact, the main muscle does not itself form an incipient sucker, but motivates a suctorial device which brings about a clamping action. Moreover, there is absolutely no evidence for a 'spring mechanism', since except for the limitations imposed by the muscular attachments, the posterior jaw of the clamp is freely hinged at its three regions of attachment to the anterior jaw.

Chauhan (1953), in a key to the families of the Diclidophoroidea, used Sproston's terms for diclidophoroidean sclerites, but accepted Price's characters in diagnosing the Diclidophoridae.

Hargis (1955), in the course of a review of the families of monogenean parasites from fishes of the Gulf of Mexico, stated that he agreed with Sproston's establishment of the homology of the clamp sclerites of the diclidophorid subfamilies Diclidophorinae and Choricotylinae with those of less modified clamps, and it is therefore unnecessary to repeat the comparisons already made above.

DISCUSSION

The present investigation of the adhesive organs of *Diclidophora* has confirmed Cerfontaine's conception of a hinged clamp operated by both intrinsic muscles and a suctorial device, and thus disagrees completely with Sproston's theory of a 'sprung' clamp operated by extrinsic muscles attached to the skeletal framework.

The comparative survey has shown that there is a basic arrangement of sclerites that is common to all species of *Diclidophora*, and this common pattern is compared in Table 2 with the pattern of sclerites in two diclidophoroideans that I have studied previously, viz. *Plectanocotyle gurnardi* and *Kuhnia scombri*. It is considered that no useful purpose would be served by speculating further upon the homologies of the various sclerites until representatives of other polyopisthocotylinean families have been examined in detail.

TABLE 2. THE CORRESPONDENCE OF SCLERITES IN THREE DICLIDOPHOROIDEAN GENERA

	Diclidophora (Present paper)	*Plectanocotyle* (Llewellyn, 1956*a*)	*Kuhnia* (Llewellyn, 1957*a*)
Peripheral sclerites			
Anterior jaw	$c_1c_2c_3$, $d_1d_2d_3$	$b_1b_2b_3$	a
Posterior jaw	ig_1g_2k	cdc	e
Median sclerites			
Anterior jaw	$a_1a_2a_3a_4$	a	cd ($+b$?)
Posterior jaw	f		

A comparison of the variations in the structure of the clamps in the different species of *Diclidophora* yields information that may be of taxonomic importance. The adhesive organ of *D. pollachii* is quite indistinguishable from that of *D. luscae*, and this observation has prompted an examination of the specific diagnoses of these two parasites. In a sample of sixteen specimens of *D. luscae* and of two specimens of *D. pollachii*, which I collected from hosts of which the identification was confirmed by an experienced student of fishes, I found the characters listed by Cerfontaine (1898) to be insufficient to separate the parasites, and my identification of the parasites rests solely upon host specificity. Under these circumstances it would be highly desirable (but very difficult) to carry out experiments on host specificity in *D. pollachii* and *D. luscae*.

The adhesive organ of *D. gadi* was found to resemble exactly that of *D. merlangi*, in size, shape, the presence of minute tubercles on the anterior jaw, and in the presence of a ring-shaped sclerotization of the diaphragm. In fact, excepting for Reichenbach-Klinke's (1951) diagnostic feature concerning the hook shape of the anterior polar filament of the egg capsule (actually it is the posterior, or ab-opercular polar filament which was illustrated as being hook-shaped), the two specimens of *D. gadi* I have examined are quite indistinguishable from *D. merlangi*. In the course of studies on larval monogeneans (Llewellyn, 1957*b*) I have observed that egg capsule formation may continue in moribund parasites, with the formation, often, of abnormally shaped capsules. It is possible, therefore, that '*Diclidophora gadi* Reichenbach-Klinke, 1951' is really *D. merlangi* from an unusual host, *Gadus aeglefinus*.

Finally, it is suggested that the development of tubercles or teeth on the anterior jaws of the adhesive organs is an indication of the closer affinity between the species of *Diclidophora* parasitizing the host genus *Gadus* (*Diclidophora minor* on *Gadus poutassou* representing the survival of the ancestral condition without tubercles) than between these species and the diclidophoran parasites on the host genera *Molva*, *Urophycis* and *Macruris*.

It is a pleasure to acknowledge the great help given to me by the Director and Staff of the Plymouth Laboratory. I am also pleased to express my gratitude to Prof. A. Brinkmann of Bergen and to Prof. O. Nybelin of Gothenburg for the loan of whole-mount preparations and serial sections of *Diclidophora minor* and *D. macruri*, to Dr H. H. Reichenbach-Klinke of Brunswick for the loan of specimens of *D. gadi*, to Dr H. M. T. Frankland for the gift of specimens of *D. denticulata* from St Andrews, and to Mr J. E. Saunders and members of the Sixth Form at King Edward's School, Five Ways, Birmingham, for collecting fresh specimens of *D. palmata* from ling off the coast of Iceland.

SUMMARY

An investigation of the adhesive mechanisms in all nine species of the genus *Diclidophora* has shown that there is a common structure consisting of a pair of hinged jaws operated by intrinsic muscles and also by a more powerful extrinsic muscle which acts on a diaphragm to produce a suction pressure that is converted into a clamping action. This investigation has revealed errors in Cerfontaine's and other descriptions of the anatomy of *Diclidophora* adhesive organs, but substantiates Cerfontaine's account of the adhesive mechanism, and therefore disagrees completely with the more recent account given by Sproston.

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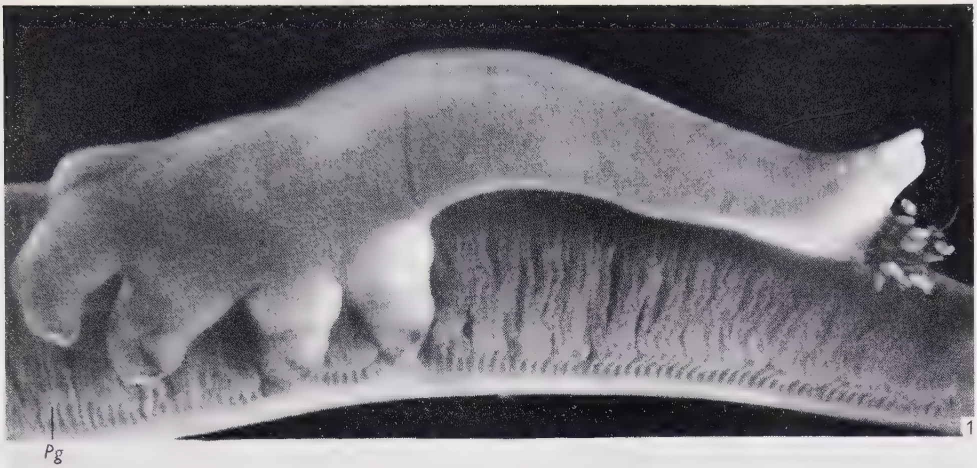

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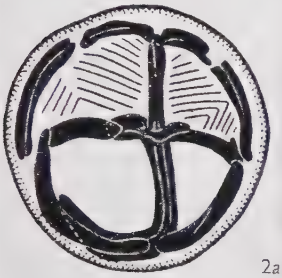

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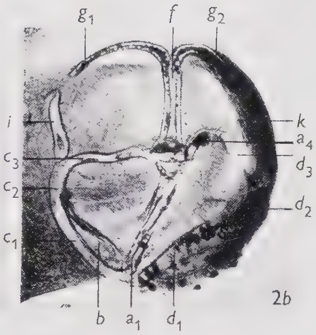
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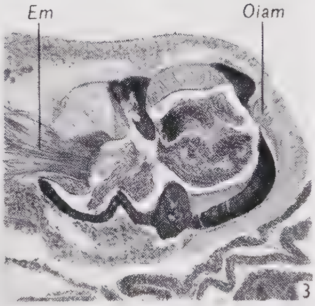

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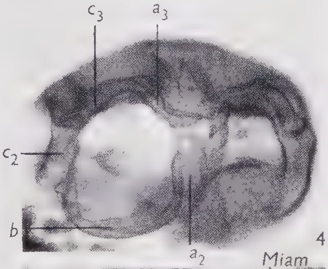

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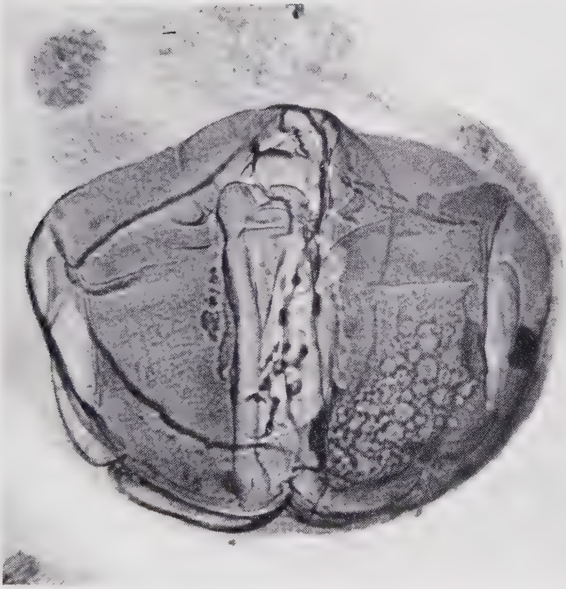

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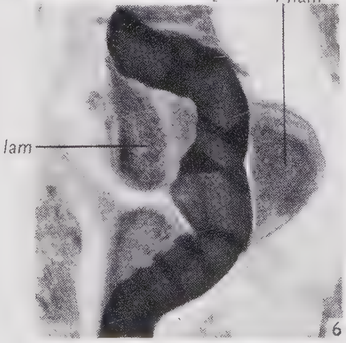

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EXPLANATION OF PLATE I

The adhesive mechanism of *Diclidophora*

Fig. 1. *Diclidophora denticulata* attached to gills of *Gadus virens*.

Fig. 2. Clamp structure in the Diclidophoridae: fig. 2*a*, after Price (1943); fig. 2*b*, clamp of *D. denticulata* forced open and seen distally for comparison with Price's diagram.

Fig. 3. Vertical section of adhesive organ of *D. palmata* parallel to the longitudinal axis of the extrinsic muscle, and obliquely through the remainder of the clamp.

Fig. 4. Adhesive organ of *D. luscae* in proximal view to show the ring which supports the diaphragm.

Fig. 5. Adhesive organ of *D. luscae*, from a flattened specimen in which sclerites of both anterior and posterior valves are seen in transparency. For interpretation see Text-figs. 1–2.

Fig. 6. Transverse section of sclerite a_2 showing the median intrinsic abductor and adductor muscles.

Abbreviations: a_1, a_2, a_3, a_4, b, c_1, c_2, c_3, d_1, d_2, d_3, f, g_1, g_2, i, k, sclerites (see text, pp. 69–71); *Em*, extrinsic muscle; *Iam*, intrinsic abductor muscles; *Miam*, median intrinsic adductor muscle; *Oiam*, outer intrinsic adductor muscle; *Pg*, proximal end of gill.

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NOTES ON THE MEDUSA *AMPHINEMA KRAMPI* RUSSELL

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(Text-figs. 1–3)

In a recent paper (Russell, 1956) I described a new medusa *Amphinema krampi* from a single specimen. I have now found three more specimens in collections made with a 2 m stramin ring trawl by R.V. *Sarsia*. Two of these were caught on 13 June 1956 at 48° 29′ N., 9° 05′ W. with 450 fathoms of wire out; one was a female 7 mm high and the other a male about 5 or 6 mm in height. The third specimen, a male 6·5 mm high, was taken on 4 July 1956 at 47° 03′ N., 5° 47′ W. with 880 fathoms of wire out. Of the three specimens the female being the best preserved was kept intact; the two males were used for sectioning.

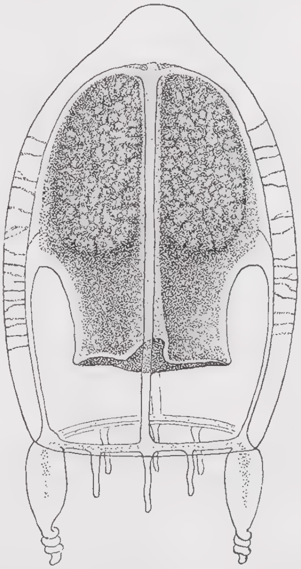
Fig. 1. *Amphinema krampi*, female, 7 mm high.

It is now possible to add to my previous description of the species. As in the specimen already described, the umbrellas are crumpled and have their margins turned inwards. In the female, however, it is certain that there is a small apical projection. The radial canals leave the stomach below the lower limits of the gonads so that there are fairly long 'mesenteries'. The marginal tentaculae are six in number in each specimen, two perradial and four interradial. It is possible therefore that the eight which I recorded in my first specimen may have been an abnormal number. The interradial female gonads are irregularly folded and much corrugated. All specimens have exactly the same coloration as that given in my previous description. A drawing of the female medusa is given in Fig. 1. The medusa bears a superficial resemblance to *Merga rubra* described by Kramp (1957, p. 14) from the Discovery Collections.

I was unable to see any discharged nematocysts so could not be certain of their type. Those on the marginal tentacles and small tentaculae are 7–9 μ

in length and appear to be micro-basic euryteles. The nematocysts along the margin of the mouth are longer, being about 13 μ in length, and may also be micro-basic euryteles.

The two males were sectioned, one longitudinally and the other transversely. Sections show that the marginal tentacles are hollow. The small marginal tentaculae are filled with endoderm cells, which are not arranged in a single row, and have endodermal roots which clasp the ring canal above and below (Fig. 2 *a–c*).

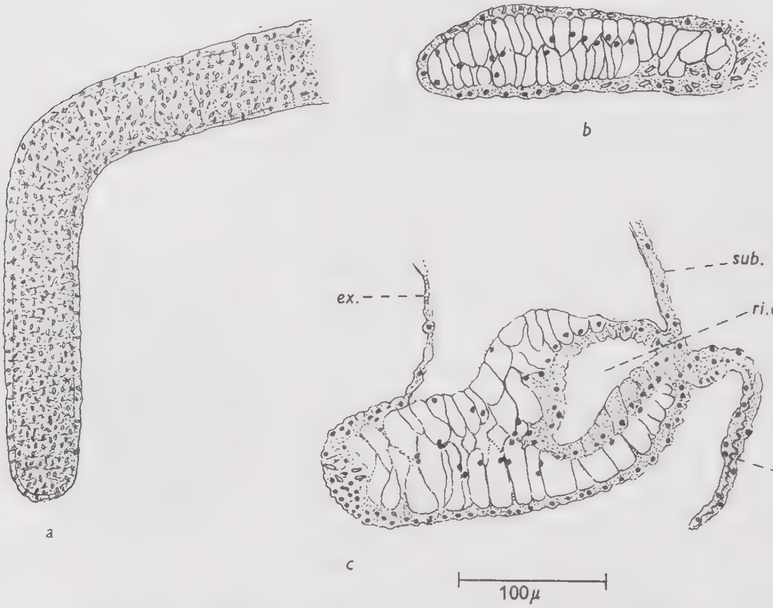

Fig. 2. *A. krampi*, small marginal tentacula. *a*, appearance of whole tentacula; *b*, *c*, sections of same, endodermal roots shown in *c*. The scale refers only to *b* and *c*. *ex*, exumbrella; *ri.c.* ring canal; *sub.*, subumbrella; *v.*, velum.

The sections afforded an opportunity to make a closer examination of the peculiar strands of tissue which run from the radial canals to the surface of the exumbrella. It should be emphasized that these medusae, which had been preserved in formalin and sea water in which they had remained for several months, were not in a good state for histological observations. Nevertheless, it was possible to observe a number of details.

There may be as many as seventeen strands in any one radius. They are distributed at intervals from about the level of the mouth well up beyond the middle of the stomach, where they occur along the lines of attachment of the stomach to the subumbrella. The strands may run through the mesogloea in rather a tortuous manner; some of them branch towards the exumbrellar

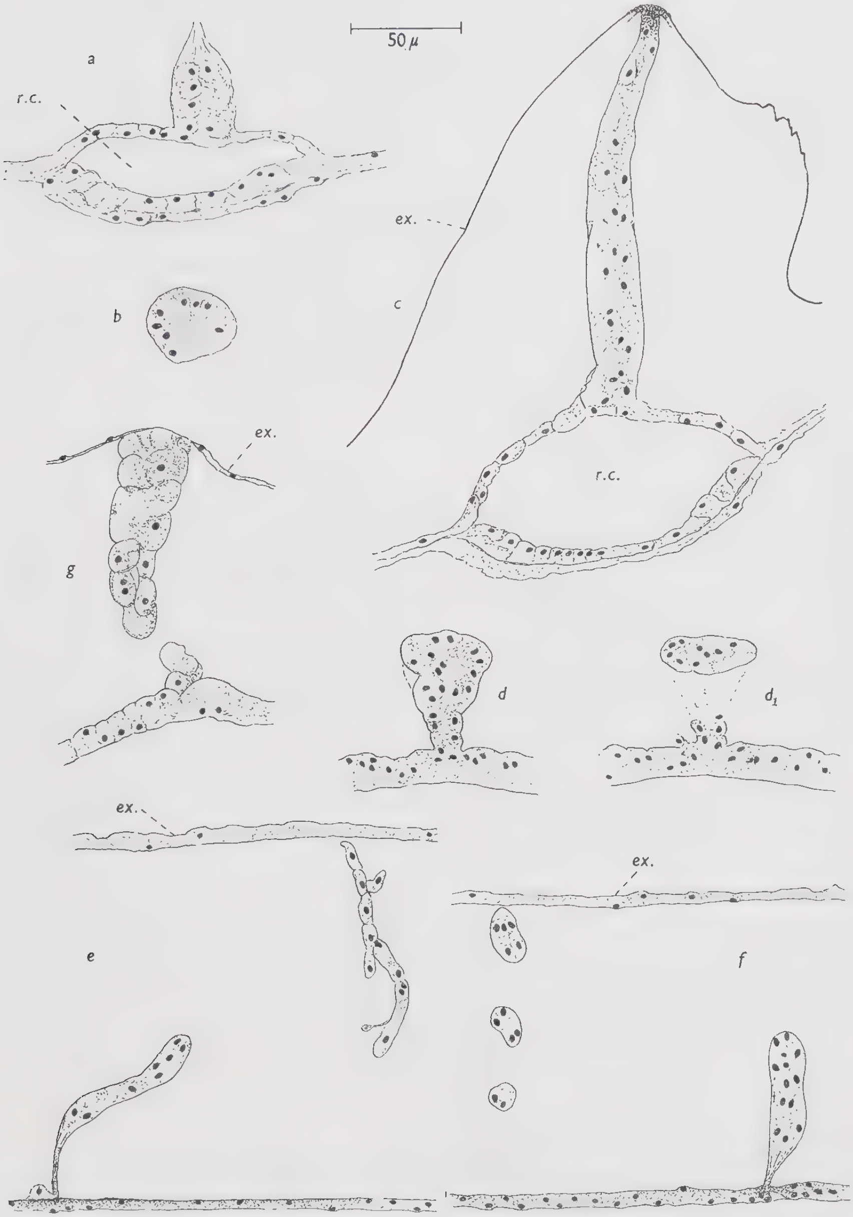

Fig. 3. Appearance of sections of cellular strands in mesogloea. *a–f*, *Amphinema krampi*; *g*, *Krampella dubia*. *a*, *b* and *c* are from transverse sections of medusae; *d*, *e* and *f* are from longitudinal sections; *d* and d_1 are consecutive sections. *ex.*, exumbrella; *r.c.*, radial canal. Camera lucida drawings, all to same scale as shown.

surface, and in one instance a single strand was seen to have two roots at its origin from the radial canal.

Most of the strands are several cells thick, but some appear to be composed of rows of single cells. The cells fill a continuous tunnel in the mesogloea, and when the root of a strand is just shaved tangentially so that no cell is included it can be seen that this hollow runs right down to the endoderm of the radial canal (Fig. 3*e*, *f*). The arrangement of cells with their nuclei round the periphery in some transverse sections (Fig. 3*b*), and the disposition of the cells in some median longitudinal sections of the roots of the strands (Fig. 3*a*, *d*), might lead one to suspect that the cells surround a duct or canal. No positive evidence could, however, be found of a true canal perforating the endoderm of the radial canal or stomach. In some sections breaks in the endoderm in the vicinity of the roots of strands are indeed to be seen, but I was not wholly convinced that these are not artifacts.

The strands end on the surface of the exumbrella in a few cells which are highly granulated (Fig. 3*c*).

I have also made a re-examination of the strands of tissue I recently recorded (Russell, 1957) in a new medusa *Krampella dubia*. Fig. 3*g* shows one of these strands as seen in a transverse section drawn to the same scale as those of *Amphinema krampi*. Although composed of fewer cells it is obviously similar in nature.

Until more and better preserved material is available it would be premature to speculate on the possible function of these unusual features, which have not been recorded in any other medusae.

I am grateful to Dr J. S. Alexandrowicz for much help he has given me, not only in the preparation of the sections, but also in their interpretation.

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THE MODES OF ACTION OF TOXIC AGENTS

III. MERCURIC CHLORIDE AND *N*-AMYLMERCURIC CHLORIDE ON CRUSTACEANS

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(Text-figs. 1–3)

In a recent study (Corner & Sparrow, 1957) it was found that differences between the toxicities of various mercury compounds to certain Crustacea were related to the resistances of the test animals to mercury poisoning, the differences being greater when a more resistant species was used. In addition, the toxicities of these poisons to a highly resistant animal were found to be closely related to the corresponding lipoid solubilities. These results were consistent with the view that Hg poisons act by penetrating the test animals, the enhanced toxicity of a highly lipoid-soluble poison such as *n*-amylmercuric chloride being the result of this compound's ability to penetrate rapidly. In the present work, tracer isotopes have been used in experiments with larvae of the brine shrimp *Artemia salina* (L.), larvae of the barnacle *Elminius modestus* Darwin and adults of the prawn *Leander serratus* (Pennant), in order to investigate further the modes of action of mercuric chloride ($HgCl_2$) and *n*-amylmercuric chloride (n-$C_5H_{11}HgCl$) as poisons to crustaceans. The results have provided evidence more direct than that obtained previously in favour of the view that penetration of the test animal is an important factor in mercury poisoning.

GENERAL METHODS

^{203}Hg-*labelled mercuric chloride*. Radioactive mercuric oxide was obtained from A.E.R.E., Harwell, and a stock solution containing 10 mg Hg/ml. was prepared by dissolving 1 g of this material in 93 ml. 50% HCl. Appropriate quantities of this solution were then added to sea water and the pH values of the solutions were adjusted to 8·1 with 2N-NaOH. To estimate the amounts of radioactivity in these sea-water solutions, samples (0·05–0·10 ml.) were first mixed with an excess of sodium sulphide to prevent volatilization of the Hg, and then evaporated to dryness on planchettes.

^{203}*Hg-labelled* n-*amylmercuric chloride*. A pure sample of this material (m.p. 121–122° C) was obtained from the Radiochemical Centre, Amersham, and a stock solution containing 0·5 mg Hg/ml. was prepared in absolute ethanol. Measured amounts of this solution were added to sea water and estimates of the radioactivity in these sea-water solutions were made on samples (0·05–0·10 ml.) slowly evaporated to dryness after treatment with an excess of reduced glutathione to prevent volatilization of the Hg compound.

Animals. For experiments in which *Artemia* and *Elminius* larvae were used the animals were obtained by methods described earlier (Corner & Sparrow, 1956). Studies were also made with the prawn *Leander serratus*, and the specimens used in these experiments were from a stock maintained at the Plymouth Laboratory.

EXPERIMENTS

UPTAKES OF MERCURIC CHLORIDE AND *N*-AMYLMERCURIC CHLORIDE BY *ELMINIUS* AND *ARTEMIA*

All the experiments were carried out at 18° C. Solutions of the poisons in sea water were prepared immediately before the experiments were started because an earlier study (Corner & Rigler, 1957) had shown that significant amounts of Hg are lost from sea-water solutions of mercuric chloride on standing. *Elminius* larvae (200–300 animals) were added to samples (50 ml.) of sea water containing 5·0, 1·0 and 0·2 mg Hg/l. as ^{203}Hg-labelled $HgCl_2$, and a second series of samples containing 0·25, 0·05 and 0·01 mg Hg/l. as ^{203}Hg-labelled $n\text{-}C_5H_{11}HgCl$. At suitable time intervals the animals were removed from the solutions by filtration on a disc of bolting silk (200 m.p.i.), suspended in plain sea water (5 ml.) and removed from the washing medium by filtration on a new disc of bolting silk. The animals were then dried by gentle suction, the sample, together with the silk, was transferred to a planchette, and its radioactivity was estimated. Control experiments showed that the bolting silk used in the first filtration was always contaminated with mercury and that this contamination was considerable on silks used to collect animals during the later stages of the experiments. It had to be taken into account in calculations. However, it was found that if the animals were washed quickly with sea water immediately after they had been removed from the toxic media they did not lose any of the Hg which they had taken up, and the disc of bolting silk used for the second filtration bore no trace of radioactivity. Accordingly, this washing procedure was used in all the experiments. After the animals in each sample had been examined for radioactivity, they were transferred to acidified sea water containing a trace of sodium azide and, as soon as all were motionless, they were counted and the quantity of Hg which they had taken up was determined as μg Hg/animal. At the end of the experiment, which was usually continued until approximately 75% of the test animals had died, the animals from all the samples were collected on a previously weighed disc of bolting silk and were dried to constant weight at 105° C. The dry weight of the animals so determined was then used to calculate the amount of Hg taken up as mg Hg/g dry wt. of larvae.

Uptake experiments were also carried out with *Artemia* larvae immersed in sea-water solutions containing 1 g and 0·5 g Hg/l., as $HgCl_2$ (10% of the mercury as ^{203}Hg) and 1·0 and 0·5 mg Hg/l. as ^{203}Hg-labelled *n*-$C_5H_{11}HgCl$. The method used in these experiments with *Artemia* was identical with that just described, except that after they had been removed from the toxic media the animals were washed twice with sea water because it was found that a single washing (as used in the procedure with *Elminius*) did not remove all traces of radioactivity from the bolting silk.

Reduced glutathione

Corner & Sparrow (1957) have shown that certain thiol compounds greatly reduce the toxicities of mercury poisons to *Elminius* and *Artemia*, and have reported that very marked effects are observed when reduced glutathione is used to protect *Elminius* from poisoning by $HgCl_2$. These workers have also demonstrated that reduced glutathione lowers the death rate of *Elminius* returned to plain sea water after a preliminary immersion in either $HgCl_2$ or *n*-$C_5H_{11}HgCl$.

Protection experiments. In the present work, by means of the methods described earlier, the rates of uptake of Hg by *Elminius* from sea water containing ^{203}Hg-labelled $HgCl_2$ (1 mg Hg/l.) and *n*-$C_5H_{11}HgCl$ (0·05 mg Hg/l.) were compared with those from similar solutions to which a tenfold excess of reduced glutathione had been added.

Recovery experiments. A study was made of the effect of reduced glutathione on the rates at which mercury was lost from poisoned *Elminius* and *Artemia* when the animals were returned to plain sea water. The procedure used was as follows. A large number of *Elminius* larvae were immersed for 20 min in sea water containing 1 mg Hg/l., and similarly *Artemia* larvae in sea water with 1 g Hg/l. Representative samples were then removed and examined immediately for radioactivity in the usual way. Simultaneously, a second series of samples were transferred to plain sea water and a third series to sea water containing reduced glutathione (10 mg/l.). The radioactivity of these animals was then estimated after they had been immersed in the two media for 1 and 5 h (*Elminius*) and 2 h (*Artemia*). Attempts were also made to repeat these experiments using ^{203}Hg-labelled *n*-$C_5H_{11}HgCl$, but these were unsuccessful because the mercury compound was insufficiently radioactive.

EXPERIMENTS WITH *LEANDER SERRATUS*

Studies were made with prawns to determine the distribution of radioactivity on and inside the test animals after they had been poisoned with ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$.

Preliminary experiments showed that prawns immersed in sea water containing 50 mg Hg/l. as $HgCl_2$ and 1 mg Hg/l. as n-$C_5H_{11}HgCl$ died in approximately the same time (3 h). The animals were, therefore, immersed in the equitoxic media for 2 h, after which time they were washed with plain sea water and various tissues, both internal (e.g. the hepatopancreas) and external (e.g. body chitin) were removed by dissection and examined for radioactivity. With chitin, pieces (*ca.* 1 cm^2) were cut from various parts of the body, dried and immediately placed under the Geiger counter. Soft body tissues, however, were first worked into a paste with ethanol and small quantities of the mixture (*ca.* 2 mg) were smeared on planchettes. The samples were then dried by slow heating and their radioactivity was estimated. As fairly large amounts of tissue were used and as ^{203}Hg is a weak emitter of β radiation it is probable that these determinations of radioactivity gave low results because of errors introduced by self-absorption. However, as exactly the same procedure was used to examine animals which had been treated either with $HgCl_2$ or with n-$C_5H_{11}HgCl$, an adequate comparison of the distributions of the two poisons was possible. After the radioactivity had been estimated the samples were dried to constant weight at 105° C and the mercury content was expressed as mg Hg/g dry wt. of tissue.

Because these experiments showed high concentrations of Hg on the gills and on the tissue under the branchiostegites of prawns poisoned with the two Hg compounds, tests were made to show whether the poison was merely attached to the surfaces of these tissues or had penetrated into them. In these experiments prawns were immersed for 2 h in sea water containing 50 mg ^{203}Hg/l. as $HgCl_2$. The animals were then removed from the solution, quickly washed with plain sea water and dissected, and the gills and branchiostegites of one of them were then examined at once for radioactivity. Gills and branchiostegites taken from one side of each of the remaining animals were immersed for 30 min in *Homarus* Ringer solution (Cole, 1941), and those dissected from the other side were immersed for the same time in *Homarus* Ringer solution containing 100 mg reduced glutathione/l. After this washing, the tissues were examined for radioactivity in the usual way.

Further experiments were carried out with prawns to show whether injecting the animals with a small quantity of a mercury poison led to a distribution of radioactivity throughout the tissues similar to that observed after the animals had been immersed in a toxic solution of the same poison. ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$ were used in these experiments, and care was taken to see that the animals received the same dose of each compound. The fact that the n-$C_5H_{11}HgCl$ had to be injected in ethanolic solution restricted the size of the dose which could be used, for in control experiments it was found that the test animals (approximately 10 g body wt.) showed signs of distress when injected with more than 0·02 ml. of ethanol. Accordingly, the quantity of each Hg compound injected was equivalent to 10 μg Hg. The needle was inserted laterally between the second and third abdominal segments and the solution was injected dorsally into the lacuna at the first segment. Immediately after the injection had been made the animals were placed in fresh sea water from which specimens were then removed at suitable intervals for dissection followed by examination of various tissues for radioactivity in the usual way.

RESULTS

EXPERIMENTS WITH *ELMINIUS* AND *ARTEMIA*

Corner & Sparrow (1957) have shown that n-$C_5H_{11}HgCl$ is 20 times as toxic as $HgCl_2$ to *Elminius* and 1000 times as toxic as this compound to *Artemia*. Interest therefore attaches to our experiments, which have shown that the rates at which *Elminius* removes mercury from sea water containing various concentrations of n-$C_5H_{11}HgCl$ are roughly the same as those at which the

TABLE 1. QUANTITIES OF MERCURY AS $HgCl_2$ AND AS n-$C_5H_{11}HgCl$ TAKEN UP BY *ELMINIUS* AND *ARTEMIA* FROM EQUITOXIC CONCENTRATIONS OF THE POISONS IN SEA WATER

(Concentrations of poisons gave TD_{50} of approximately 3 h with each species.)

	$HgCl_2$		n-$C_5H_{11}HgCl$	
Animal	Concn. in toxic medium (mg Hg/l)	Concn. taken up by animals (mg Hg/g dry wt.)	Concn. in toxic medium (mg Hg/l)	Concn. taken up by animals (mg Hg/g dry wt.)
Elminius	0·2	0·92	0·01	0·70
Artemia	1000	0·47	1·0	0·28

TABLE 2. INFLUENCE OF REDUCED GLUTATHIONE ON THE LOSS OF MERCURY BY *ELMINIUS* AND *ARTEMIA* POISONED WITH $HgCl_2$

(*Elminius* immersed in sea-water solution of $HgCl_2$ (1 mg Hg/l.) for 20 min. *Artemia* immersed in sea-water solution of $HgCl_2$ (1 g Hg/l.) for 90 min. GSH = reduced glutathione (10 mg/l.).)

	Washing medium			
	Sea water. Concn. of Hg in animals		GSH in sea water. Concn. of Hg in animals	
Time (h)	mg Hg/g dry wt.	%	mg Hg/g dry wt.	%
	Elminius			
0	0·54	100	0·54	100
1	0·35	65	0·35	65
5	0·16	29	0·18	33
	Artemia			
0	0·52	100	0·52	100
2	0·33	63	0·27	52

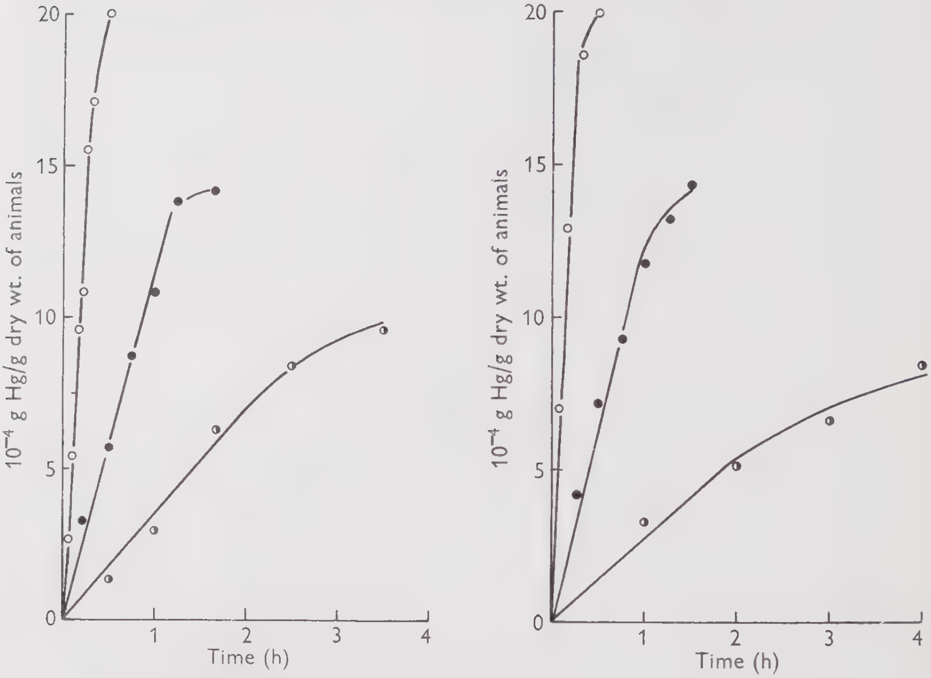

Fig. 1. Left: uptake of mercury as mercuric chloride by *Elminius* larvae. ○—○, 5 mg Hg/l. (TD_{50} = 0·55 h); ●—●, 1 mg Hg/l. (TD_{50} = 1·1 h); ◑—◑, 0·2 mg Hg/l. (TD_{50} = 2·9 h). Right: uptake of mercury as *n*-amylmercuric chloride. ○—○, 0·25 mg Hg/l. (TD_{50} = 0·6 h); ●—●, 0·05 mg Hg/l. (TD_{50} = 0·9 h); ◑—◑, 0·01 mg Hg/l. (TD_{50} = 3·0 h).

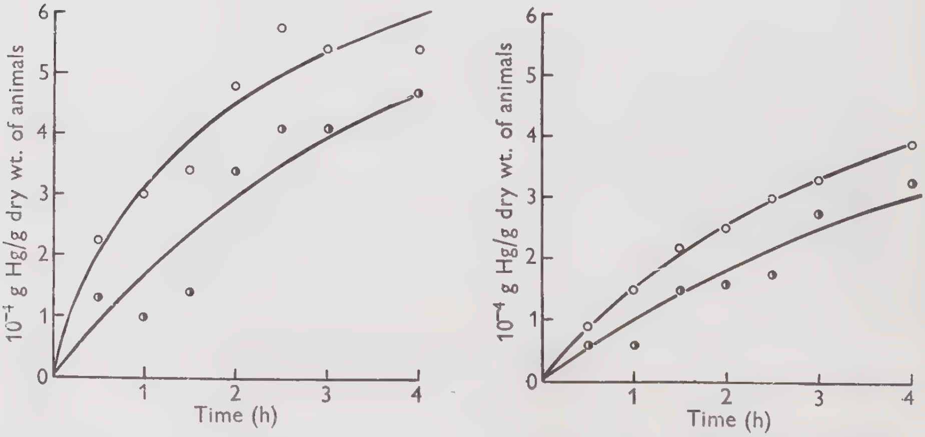

Fig. 2. Left: uptake of mercury as mercuric chloride by *Artemia* larvae. ○—○, 1 g Hg/l. (TD_{50} = 2·3 h); ◑—◑, 0·5 g Hg/l. (TD_{50} = 3·6 h). Right: uptake of mercury as *n*-$C_5H_{11}HgCl$. ○—○, 1 mg Hg/l. (TD_{50} = 2·5 h); ◑—◑, 0·5 mg Hg/l. (TD_{50} = 3·5 h).

animal takes up Hg from sea water containing 20 times each concentration as $HgCl_2$ (Fig. 1). Similar experiments with *Artemia* (see Fig. 2) have shown that the rates at which this animal concentrates mercury as $n\text{-}C_5H_{11}HgCl$ are approximately the same as those at which it takes up the poison from sea water containing 500 times each concentration as $HgCl_2$. The correlation between toxicity and uptake data is, therefore, less exact for *Artemia*. Nevertheless, it may be concluded that differences between the toxicities of the two poisons reflect to a considerable extent differences between the rates at which the compounds are taken up by both species of test animal.

The results shown in Table 1 draw attention to the vast difference between the concentrations of each Hg compound which must be added to the surrounding medium in order to kill *Artemia* and *Elminius* at the same rate. Thus, the concentrations of $HgCl_2$ and $n\text{-}C_5H_{11}HgCl$ which are equitoxic to *Artemia* and *Elminius* are in the ratio 5000:1 and 100:1 respectively. Of greater interest, however, is the finding that although much higher external concentrations are needed to kill *Artemia*, the actual quantities of mercury which these animals accumulate by the time 50% have died are significantly less than the corresponding amounts taken up by *Elminius*.

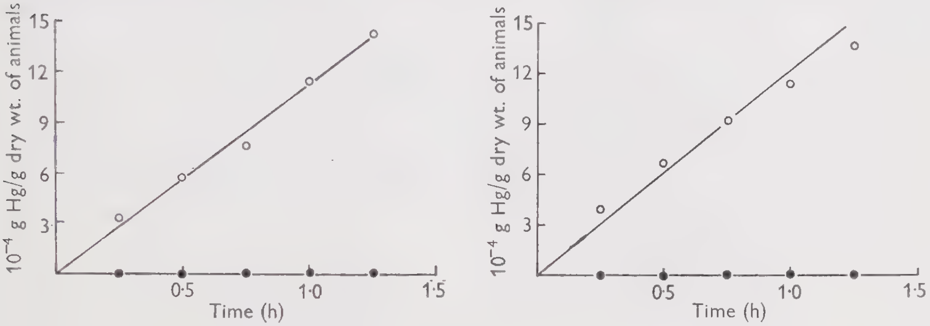

Fig. 3. Left: uptake by *Elminius* larvae of mercuric chloride (1 mg Hg/l.), alone (○—○) and in the presence of a tenfold excess of reduced glutathione (●—●). Right: uptake of $n\text{-}C_5H_{11}HgCl$ (0·05 mg Hg/l.) alone (○—○) and in the presence of a tenfold excess of reduced glutathione (●—●).

The results of experiments in which reduced glutathione was used to protect *Elminius* from the toxic effects of $HgCl_2$ and $n\text{-}C_5H_{11}HgCl$ (see Fig. 3) showed that the addition of a tenfold excess of the thiol compound to the toxic media completely prevented any uptake of mercury by the test animals during the time in which more than 75% of the unprotected animals died (3 h). When further experiments were carried out to examine the influence of reduced glutathione on the rates of loss of mercury by *Elminius* and *Artemia* previously poisoned with $HgCl_2$ and $n\text{-}C_5H_{11}HgCl$ it was found that the thiol compound had little or no effect on these very slow processes (see Table 2).

EXPERIMENTS WITH *LEANDER*

Findings made in experiments with prawns were of considerable interest because they demonstrated that animals killed by immersion in toxic solutions of $HgCl_2$ and n-$C_5H_{11}HgCl$ contained significant amounts of Hg in several internal organs (see Table 3). Moreover, after the animals had been killed by immersion for 2 h in equitoxic solutions of the two poisons the patterns of distribution of the Hg throughout the animals were markedly different. Thus, when tissues in contact with the surrounding medium were examined it was found that whereas $HgCl_2$ concentrated in the tissue beneath the branchiostegites, n-$C_5H_{11}HgCl$ was detected primarily in the gills. The distributions of the poisons inside the test animals also differed in that whereas $HgCl_2$ concentrated almost exclusively in the antennary gland, n-$C_5H_{11}HgCl$ was found in roughly equal amounts in this organ and the thoracic ganglionic mass.

TABLE 3. AMOUNTS OF MERCURY FOUND IN THE TISSUES OF PRAWNS IMMERSED IN EQUITOXIC SOLUTIONS OF $HgCl_2$ AND n-$C_5H_{11}HgCl$

(Concentrations of poisons: $HgCl_2 \equiv 50$ mg Hg/l.; n-$C_5H_{11}HgCl \equiv 1$ mg Hg/l. $TD_{50} \simeq 3$ h.)

	mg Hg/g dry wt. tissue	
Tissue	$HgCl_2$	n-$C_5H_{11}HgCl$
External		
Branchiostegite	4·3	1·6
Pleopods	0·48	0·86
Dorsal chitin	0·13	0·17
Gills	0·49	2·23
Internal		
Antennary gland	0·32	0·46
Hepatopancreas	0·02	0·05
Central nervous system	0·04	0·38
Muscle	0·00	0·04

By contrast, it was found that after the animals had been injected with sublethal doses of $HgCl_2$ and n-$C_5H_{11}HgCl$ the patterns of distribution of the two poisons were similar in that both compounds were found to concentrate in the gills and antennary glands of the test animals (see Table 4).

Further experiments using reduced glutathione gave results consistent with those obtained in similar studies with *Elminius* and *Artemia*. Thus, no significant amount of mercury was removed when the gills of prawns previously treated with mercuric chloride were washed either with *Homarus* Ringer alone or fortified with reduced glutathione. In addition, the branchiostegites of these animals were found to lose less than half their Hg content when washed with either medium (see Table 5). Because these tissues are in continuous contact with the surrounding medium and were found to accumulate far more Hg than any other tissue, it seemed reasonable to expect that if Hg acted by simply becoming attached to the surfaces of the test animals these would be the most likely sites of attachment. However, it appeared from these findings that all the mercury accumulated by the gills and most of that taken up by the tissue under the branchiostegite was not attached to the surfaces of these tissues, but had penetrated into them.

TABLE 4. AMOUNTS OF MERCURY DETECTED IN THE TISSUES OF PRAWNS INJECTED WITH MERCURIC CHLORIDE AND *n*-AMYLMERCURIC CHLORIDE

(Concentrations used: 10 μg Hg/l animal injected in 0·01 ml. sea water ($HgCl_2$) and 0·02 ml. ethanol (n-$C_5H_{11}HgCl$).)

Tissue	μg Hg/g dry wt. tissue	
	$HgCl_2$	n-$C_5H_{11}HgCl$
External		
Branchiostegite	13·0	4·3
Pleopods	2·2	1·0
Dorsal chitin	3·4	0·2
Gills	29·3	22·0
Internal		
Antennary gland	13·3	6·7
Hepatopancreas	4·4	4·2
Central nervous system	3·5	3·0
Muscle	2·7	0·0

TABLE 5. THE REMOVAL OF MERCURIC CHLORIDE FROM THE GILLS AND BRANCHIOSTEGITES OF PRAWNS POISONED WITH THIS COMPOUND

Washing medium	Counts/min/mg dry wt. of tissue	
	Gills	Branchiostegite
None	100	1795
Homarus Ringer	118	1160
Homarus Ringer (plus 100 mg reduced glutathione/l.)	102	1150

DISCUSSION

In earlier studies, Corner & Sparrow (1956, 1957) showed that great differences are found between the susceptibilities of *Artemia* and *Elminius* to mercury poisons; that differences between the toxicities of certain organomercury poisons and that of mercuric chloride are far greater when a highly resistant test animal like *Artemia* is used; that, in general, poisons which are very toxic are also highly lipoid-soluble; and that no correlation exists between the toxicities of these compounds and their abilities to inactivate enzymes. Corner & Sparrow considered these observations to be consistent with the view that Hg poisons act by penetrating the test animal and assumed that the extreme resistance of *Artemia* was a direct result of this animal's impermeability. Their findings did not, however, exclude the possibility that the large differences between the susceptibilities of *Artemia* and *Elminius* to Hg poisons were not because *Artemia* accumulated the compounds at a much slower rate, but because this animal was able to tolerate a much higher concentration of mercury in its tissues. This possibility has therefore been examined in the present work, and it has been found that both $HgCl_2$ and n-$C_5H_{11}HgCl$ are, in fact, accumulated much more rapidly by *Elminius* than by *Artemia*. Moreover, the amount of Hg, as either compound, taken up by *Artemia* at a time when 50% of the test animals die, is significantly less than the corresponding amount accumulated by *Elminius*. It therefore seems clear that *Artemia* is very resistant to Hg compounds, not because the tissues of this animal are able to withstand large concentrations of mercury, but because this animal accumulates the poisons at a very slow rate.

Further results of the present work were that both *Artemia* and *Elminius* take up n-$C_5H_{11}HgCl$, which is very toxic and very lipoid-soluble, at a rate much faster than that at which they accumulate $HgCl_2$; and that the relative rates of uptake of the two poisons are related to their relative toxicities. However, although these findings emphasized that high toxicity attends a high rate of accumulation by the test animal, they did not demonstrate how this accumulation took place, and the possibility could not be ignored that the organomercury compound might be taken up more rapidly than $HgCl_2$ because of preferential adsorption on the animal's surface. It is true that Corner & Sparrow (1957) have provided evidence in favour of the view that as far as interaction with proteins present on the surface of the test animal is concerned, n-$C_5H_{11}HgCl$ is likely to be less effective than $HgCl_2$, but in the present work it was thought desirable to seek more direct evidence that penetration of the test animal took place. Accordingly, semi-quantitative studies were made with *Leander*, an animal large enough to permit excision of various internal organs which could be examined for their Hg content. In these experiments it has been found that when the animals are immersed in equitoxic solutions of the two poisons, significant amounts of Hg can, in fact, be detected in their internal tissues. Moreover, these amounts are slightly greater for n-$C_5H_{11}HgCl$, although the concentration of this poison used in the external medium is equivalent to only one-fiftieth of the quantity of Hg used as $HgCl_2$. These experiments, therefore, provide direct evidence that Hg compounds can penetrate a crustacean and that an organomercury poison enters the test animal at a rate much faster than that of $HgCl_2$. The poisons have also been found in considerable quantity on external sites such as the gills and branchiostegites, on which they might be in a position to exert at least part of their toxic action. Further experiments, however, have provided evidence in favour of the view that attachment to the surface is not of primary importance for the toxic action of either poison. Thus, if the compounds act simply by becoming attached to these surfaces it seems likely that their toxic effects would be readily reversed by substances such as reduced glutathione, because the marked affinities of thiol compounds for heavy metals (cf. Gurd, 1954) have often been exploited as a means of removing these poisons from biological surfaces. During toxicity studies with *Elminius* and *Artemia*, Corner & Sparrow (1957) found that thiol compounds effectively protect the test animals against mercury poisons, and in the present work it has been found that reduced glutathione can prevent *Elminius* from accumulating mercury either as $HgCl_2$ or as $n\text{-}C_5H_{11}HgCl$. These results support the view that the affinity of the thiol compound is greater than that of the surface of the test animal for either poison. Because of this it might be expected that reduced glutathione would readily remove these poisons from the surface of *Elminius*, and some evidence of this was obtained from further experiments by Corner & Sparrow in which it was found that reduced glutathione lowers the death rates of *Elminius* transferred to plain sea water after preliminary treatments with mercury poisons. However, these experiments lasted a long time and it is possible that the thiol compound did not remove the poisons from the surfaces of the test animals but penetrated into them and inactivated a proportion of the mercury poison after it had reached the tissues. The results of shorter experiments carried out in the present work have shown that reduced glutathione has little or no influence on the very slow rates of loss of mercury by either *Elminius* or *Artemia* previously poisoned with mercuric chloride, nor does it influence the loss of mercury from the surface tissues (e.g. gills and branchiostegites) of prawns poisoned with this compound. These findings therefore lend support to the view that the Hg compound was not simply attached to the surfaces of the test animals, but penetrated into them.

Examination of the patterns of distribution of $n\text{-}C_5H_{11}HgCl$ and $HgCl_2$ injected into prawns has given further information concerning the origin of the mercury detected on the gills and branchiostegites, for it has been found that these tissues also contain large quantities of Hg after the animals have been injected with either poison. This finding suggests that the Hg detected on these surfaces after the animals have been immersed in toxic solutions of the poisons might not arise exclusively as the result of direct accumulation from the surrounding medium, but might to some extent represent an attempt by the animal to excrete through these tissues some of the assimilated Hg. On this point it is interesting that Hg poisons have been found to concentrate in the antennary gland of the prawn after the animals are immersed in toxic solutions, and after they have been given a sublethal dose of each compound by injection, for the decapod antennary gland is known to be associated with excretory mechanisms. A further point of interest is that $HgCl_2$ has been found to affect osmoregulation in *Daphnia magna* (Holm-Jensen, 1948).

There are obvious dangers in attempting to base a theory of Hg poisoning on the results of studies made with several different test species, but from the findings just discussed it appears that penetration of the animal and subsequent interference with excretory mechanisms is a useful working hypothesis on which to base future studies of the modes of action of Hg compounds as poisons to crustaceans.

During this work one of us (F. H. R.) was supported by a Research Fellowship from the National Research Council of Canada. The other is indebted to International Paints Ltd. for a Research Fellowship, and both gratefully acknowledge the assistance of this firm in the purchase of radioactive materials. The research facilities provided by the Plymouth Laboratory of the Marine Biological Association are also gratefully acknowledged.

SUMMARY

Experiments have been carried out with ^{203}Hg-labelled *n*-amylmercuric chloride (n-$C_5H_{11}HgCl$) and mercuric chloride ($HgCl_2$) to study the modes of action of these compounds as poisons to some crustaceans.

Differences between the susceptibilities of *Artemia salina* and *Elminius modestus* to the poisons do not reflect differences between the quantities of these compounds which the animals can tolerate in their tissues, but are directly related to the rates at which the poisons are accumulated. Thus, experiments with either species have shown that Hg is taken up at approximately the same rate from equitoxic solutions of the two poisons; and the rates at which Hg is accumulated by the two species from equitoxic solutions of either poison are of the same order.

Experiments with reduced glutathione have given results consistent with the view that most of the Hg taken up by either species penetrates into the tissues of the test animals and does not act simply by becoming attached to their surfaces.

Direct evidence of the penetration of Hg compounds into a crustacean has been obtained from experiments with *Leander serratus*. Considerable amounts of Hg have been detected in the antennary glands.

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OBSERVATIONS ON *COCHLODESMA PRAETENUE* (PULTENEY) [EULAMELLIBRANCHIA]

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(Text-figs. 1–12)

Cochlodesma praetenue is the sole British species of the family Laternulidae included in the suborder Anomalodesmata (Thiele, 1935). Shell characters clearly distinguish this suborder from the remainder of the Eulamellibranchia. These characters include a thin, fragile, white shell that is usually inequivalve, hinge teeth that are weak or absent and a ligament that is well developed, complex and may include a lithodesma. Further, Yonge (1952) and Allen (1954) showed that the shell valves may be united dorsally by the periostracum and that some members of the group incorporate sand grains in the periostracum. Work by Ridewood (1903) and Atkins (1937*a*, *b*) and more recently by Yonge (1952) and Allen (1954) on the anatomy of members of the Anomalodesmata also shows other distinguishing characters, notably in the form of the ctenidia and of the stomach.

Knowledge of the functional morphology of the suborder is confined to the families Lyonsiidae, Pandoridae and Thraciidae. When *C. praetenue* was found to be obtainable in numbers from the Northumberland coast, advantage was taken to examine a member of another family of the Anomalodesmata and to compare it with related forms.

HABITAT

Cochlodesma praetenue is distributed from Iceland and the southern part of Norway to the Mediterranean (Forbes & Hanley, 1853). It is recorded from all British waters except the southern North Sea. It occurs in fine gravel, sand and muddy sands from extreme low-water mark of spring tides to a depth of 60 fathoms. It is most commonly found in sand and sandy-gravel in sheltered conditions just below the level of low water of spring tides. Specimens for this present study were taken in such a habitat from a beach at Low Newton on the Northumberland coast.

The common species of the associated infauna were: *Nephthys hombergi* Lam., *Lanice conchilega* (Pallas), *Ampelisca brevicornis* (A. Costa), *Tellina fabula* Gmelin, *Venus striatula* da Costa, *Phacoides borealis* (Linné), *Ensis siliqua* (Linné) and *Echinocardium cordatum* (Pennant). *Cochlodesma* is buried to a depth of 7 cm below the surface of the substratum and usually lies in a horizontal plane. Allowed to burrow in its normal substratum in aquarium tanks the animal comes to lie horizontally on either valve, slight preference being shown for the left side to be uppermost.

MORPHOLOGY AND FUNCTIONS

Mantle/shell

The thin shell valves are white and slightly inequivalve. The right valve is more convex than the left and overlaps it slightly. The margins of the valves are sinuate (Fig. 1). Except for an area posterior to the umbonal ridge the shell is smooth. Posterior to the umbonal ridge the shell is roughened by numerous small pustules. Fine sand grains adhere to the roughened surface and make the shells conspicuous in sievings of sand samples. Unlike *Lyonsia* and *Pandora* the grains are not incorporated within the periostracum but lie on the surface and are kept in position by the roughness alone.

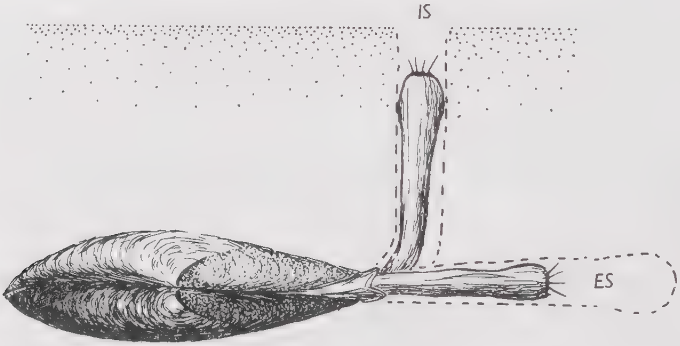

Fig. 1. *Cochlodesma praetenue* in its normal position with the siphons extended. *ES*, exhalant siphon; *IS*, inhalant siphon.

Hinge teeth are not present. The ligament is complex and unusual, and is composed of an internal and an external portion. The internal portion of the ligament is held between a pair of spoon-shaped extensions of the shell which are carried at the end of a pronounced internal ridge that runs forward from the anterior edge of the posterior adductor muscle scar (Fig. 2). The internal portion of the ligament is wedge-shaped and is composed of three layers. The latter probably correspond with the outer, inner and fusion layers of Owen, Trueman & Yonge (1953) and are referred to as such in the following account. The tip of the wedge connects with the external part of the ligament which, apart from a pair of small wing-like extensions of the inner layer, is composed entirely of fusion layer and its covering of periostracum (Fig. 3 A, B). This external part extends for some distance anterior and posterior to the umbones and at the junction of the internal and external portions there is a lateral extension of the fusion layer that follows the line of the umbonal ridge

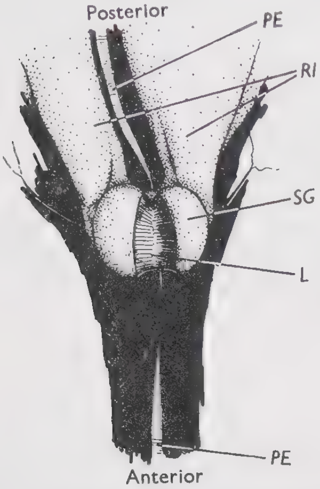
Posterior
PE
RI
SG
L
PE
Anterior

Fig. 2. The hinge mechanism as seen from the inside of the valves showing the terminal spoon-shaped extensions (*SG*) of the ridge (*RI*) holding the ligament (*L*). The valves are united dorsally by the periostracum (*PE*).

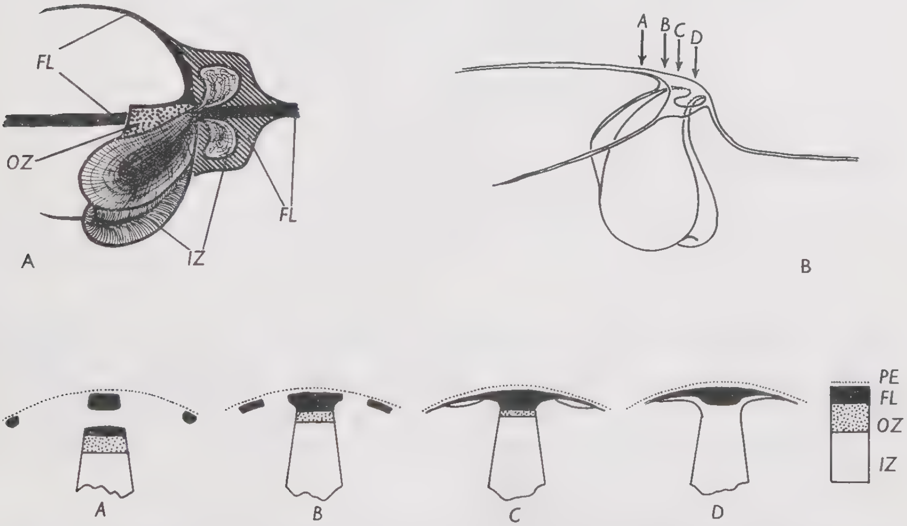
FL
OZ
FL
IZ
A
A
B
C
D
B
A
B
C
D
PE
FL
OZ
IZ

Fig. 3. A. The ligament as seen from the ventral side. The inner portion has been inclined to the side. *FL*, fusion layer; *IZ*, inner layer; *OZ*, outer layer. B. Outline drawing of the ligament in side view with arrows indicating the position of four transverse sections, *A*, *B*, *C* and *D*. The latter are drawn diagrammatically. Lettering as before.

(Fig. 3 A, B). Dorsally the fused periostracum extends beyond the limits of the fusion layer as far as the anterior and posterior adductor muscles and parallels the condition in the Lyonsiidae and the Pandoridae.

The mantle edges are extensively fused. The fusion involves the inner mantle fold and the inner surface of the middle fold in the manner described by Yonge (1957). The pedal aperture occupies little more than a third of the ventral margin. There is no fourth pallial aperture present, and in this respect *Cochlodesma* resembles *Pandora* rather than *Thracia* and *Lyonsia*. Unlike those of *Pandora*, the periostracal grooves are well defined, and sand grains do not adhere to the newly secreted periostracum.

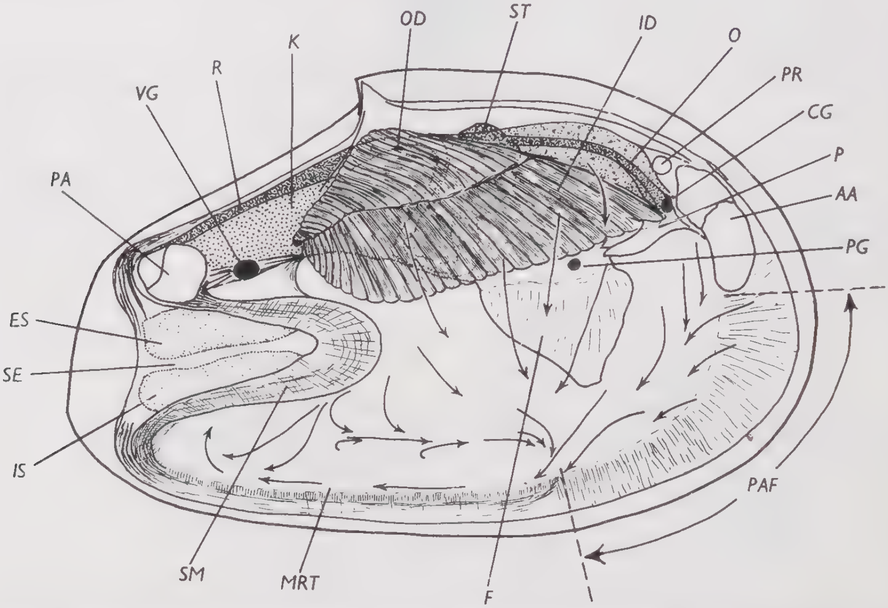

Fig. 4. Lateral view of *Cochlodesma praetenue* with the right valve removed. The ciliary currents of the mantle surface are indicated by arrows. *AA*, anterior adductor muscle; *CG*, cerebral ganglion; *F*, foot; *ID*, inner demibranch; *K*, kidney; *MRT*, main rejection tract; *O*, oesophagus; *OD*, outer demibranch; *P*, palps; *PA*, posterior adductor muscle; *PAF*, pedal aperture; *PG*, pedal ganglion; *PR*, pedal retractor muscle; *R*, rectum; *SE*, siphonal embayment; *SM*, siphonal retractor muscles; *ST*, stomach; *VG*, visceral ganglion. Other lettering as before.

The siphons are well developed and separate and can be retracted into an extensive siphonal embayment (Figs. 1, 4). They are formed from the inner fold of the mantle and can expand to more than twice the length of the shell. Each siphon can expand and contract independently of the other. Separate mucus-lined tubes are formed by the siphons. The tubes are similar to those of *Thracia* (Yonge, 1937), but whereas in *Thracia* both tubes open to the surface only the inhalant tube does so in *Cochlodesma*. The exhalant tube extends horizontally into the substratum for a distance approximately equal to the depth the shell is buried. The mucous linings are less robust than those of *Thracia* and a complete tube was never separated from the substratum. Inflation of the distal ends of the siphons is not as marked as in the case of *Thracia*. Some inflation occurs for the purpose of consolidating the mucous secretion. Observations on tubes built against the sides of glass containers show that on completion of the tube the siphons do not completely retract, but remain extended for a few millimetres beyond the valve margins. No further extension of the siphon occurs except for the repair of the tube. Under experimental conditions *Cochlodesma* does not remain in the same position for long periods of time, new tubes being built every 12–72 h. This differs from the Lucinacea (Allen, 1953) which also build a mucus-lined tube yet remain in the same position for considerable periods of time. Unlike *Thracia* (Yonge, 1937), there is no concentration of mucus-secreting cells at the distal end of the siphons, secretion taking place along their whole length in *Cochlodesma*.

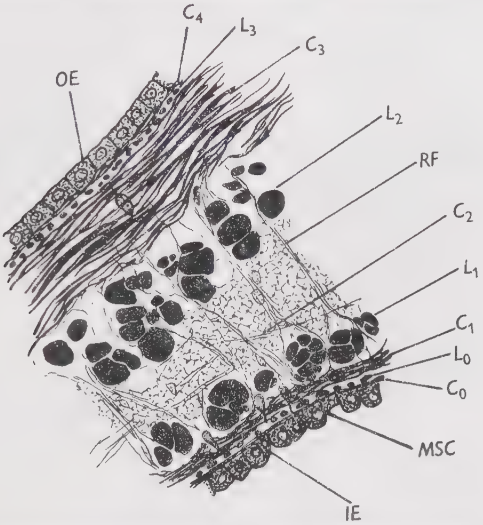

Fig. 5. Transverse section through a siphon to show the arrangement of the various layers. C_0–C_4, circular muscle layers; *IE*, inner epithelium; L_0–L_3, longitudinal muscle layers; *MSC*, mucus-secreting cells; *OE*, outer epithelium; *RF*, radial muscle fibres.

The siphons elongate smoothly and expand and contract when the terminal aperture is either open or closed. No definite pattern of movement was observed. It appears that expansion is not necessarily a result of pressure on the water in the mantle cavity, a method described for *Mya* (Chapman & Newell, 1956). The histology of the siphons of *Cochlodesma* is similar to that of the Tellinacea (Yonge, 1949; Chapman & Newell, 1956) (Fig. 5), and they expand in a way similar to that described by Chapman & Newell for *Scrobicularia plana*. Trichrome staining shows that there is much more circular muscle and less collagen in *Cochlodesma* than in the latter species and no lattice work of collagen was demonstrated. In Fig. 5 the nomenclature of the various muscle layers corresponds with that given by Yonge (1949) for the Tellinacea, the only differences being the presence of additional layers L_0 and C_0. C_2 is not well defined and a parenchymatous packing tissue fills the space between layers L_1 and L_2. Thus, the arrangement of the muscle layers is the same on either side of the central packing tissue. Well-defined nerve strands, four in the inhalant and six in the exhalant siphon, run through the central tissue, and supply four to eight simple tentacles at the aperture edge.

It is possible to correlate siphon structure in lamellibranchs with differences in the habits of the animals. Yonge (1949) relates the great activity of the siphons in deposit-feeding Tellinacea with the separation and arrangement of the various layers, and shows that in suspension feeders such as the Veneracea (and *Mya*) there is no marked differentiation into layers. *Cochlodesma* is undoubtedly a suspension feeder, yet has siphons with well developed and intricate muscle layers similar to the Tellinacea. This apparent anomaly must be related to the activity required of the siphons in building the mucus-lined tubes. Furthermore, the lack of collagen with the corresponding increase in the circular musculature must be related to the tube-building function and the periodic need to increase the diameter of the siphon. As Chapman & Newell (1956) point out, collagen is inextensible and limits any increase in the diameter of the siphons.

The direction of the ciliary currents on the mantle are as shown in Fig. 4. With the exception of particles carried in the anterior currents, which may pass on to the surface of the palps, all are rejected. The particles are carried to a point close to the posterior limit of the pedal aperture, and from there posteriorly along the main rejection tract on the fused inner mantle folds to a point ventral to the inhalant aperture. Unlike *Pandora*, there is no mantle fold forming a roof over the main rejection tract. Apart from scattered mucus-secreting cells, the only concentration of glandular tissue in the mantle is close to the posterior apertures over the area of insertion of the siphonal retractor muscles. The elongate cells are modified epidermal cells and it is probable that their secretion is used in the formation of the pseudofaeces.

Viscera

The ciliary currents over the surface of the body are concerned with the rejection of particles. There is a main tract from the palps to the heel, and particles from the remainder of the body surface move posteriorly towards this tract. Apart from a narrow strip at the ventral edge, the foot is not ciliated (Fig. 6).

The viscera can be seen through the body wall. Gonads and digestive diverticula are compact and, as in most members of the Anomalodesmata, *Cochlodesma* is hermaphrodite. The ovaries lie posterior to the long axis through the style sac and stomach, the digestive diverticula being anterior to this line. The testes are placed between the digestive diverticula and the musculature of the foot. The course of the alimentary canal is also seen through the body wall as shown in Fig. 6. The kidney, although elongate, is similar to that of other Eulamellibranchia. The large visceral ganglion, instead of lying close to the posterior adductor muscle, is some distance anterior to the muscle below the kidney. This is probably correlated with the considerable depth of the siphonal embayment, the siphonal nerve having a short and direct route between the ganglion in this position and the base of the siphon.

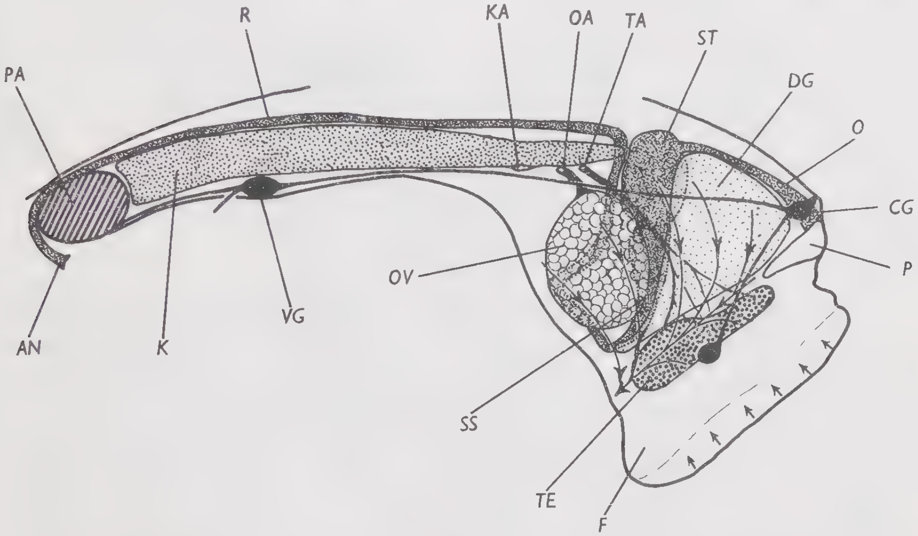

Fig. 6. View of the right side of the body to show the arrangement of the viscera and the direction of the ciliary currents on the body surface. *AN*, anus; *DG*, digestive diverticula; *KA*, renal aperture; *OA*, female genital pore; *OV*, ovary; *SS*, style sac; *TA*, male genital aperture; *TE*, testis. Other lettering as before.

Gills

The gills are typical of the Anomalodesmata with the outer demibranch reduced and reflected dorsally. They are deeply plicate (9 filaments/plica). Occasional specimens have the first 15–20 filaments non-plicate. The ciliary sorting mechanisms are of the *Pinna* type (Atkins, 1937*a*). All particles reaching the gill surface are carried to the marginal groove of the inner demibranch. Atkins (1937*a*, p. 349) figures the margin of the inner demibranch of *Cochlodesma praetenue* and the present work confirms her observations. The terminal frontal cilia are not modified to form a sorting device of the type described in *Pandora* (Allen, 1954). The gills are short and terminate anterior to the posterior adductor muscle (Fig. 4).

Palps

The palps are strap-shaped and small. The sorting folds of the inner surface are relatively broad because of the unusual breadth of the shelf on the aboral side of the fold (Fig. 7). The ciliation shows no significant difference from the basic eulamellibranch type and the palps function in the typical manner.

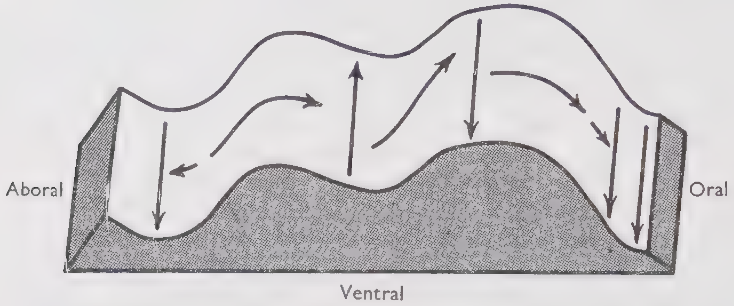

Fig. 7. Diagrammatic section of a palp fold to show the direction of ciliary beat.

Alimentary canal

The course of the alimentary canal is indicated in Fig. 6. The mouth opens into an unusually long oesophagus that passes along the dorsal edge of the digestive diverticula and opens at the head of the stomach. The lumen of the oesophagus is dorso-ventrally compressed with deep dorsal, ventral and lateral grooves. The stomach is large with its long axis lying at right angles to that of the oesophagus, i.e. dorso-ventrally. The mid-gut and style sac are combined and open from the posterior end of the stomach. The intestine makes a simple loop behind the stomach and passes dorsally over the kidney and the posterior adductor muscle to open at the anus.

The stomach was studied in some detail as earlier work on the related genus *Pandora* (Allen, 1954) showed differences from the stomachs of other eulamellibranchs. By dissection and serial sections the morphology and ciliary currents of the stomach were investigated. The nomenclature of Owen (1953, 1955) has been followed.

The stomach of *Cochlodesma* is similar to that of *Pandora* (Figs. 8, 9. Distortion in Fig. 8 has been confined largely to the gastric shield). Accepted particles entangled in a string of mucus are pulled into the stomach by the capstan-like action of the style. The average time taken for one revolution of the style was recorded for twenty-one specimens. In three (at 16·5° C) the time for one revolution was 3·2 sec. In the remaining eighteen specimens (at 17° C) the time varied from 2·6 to 4·9 sec/rev., eleven records lying between 3·2 and 3·8 sec/rev. The style rotates in a clockwise direction when viewed from the oesophageal end of the stomach. It is prevented from entering the dorsal hood by the tooth of the gastric shield (Fig. 10). Typically the shield is held in position by flanges penetrating the left pouch and the dorsal hood. The morphology and ciliary currents are basically similar to those of *Pandora* (Allen, 1954). In *Cochlodesma* the dorsal hood is well developed. The acceptance tract, terminating in a pad of tissue anterior to the end of the minor typhlosole, is considerably wider than that of *Pandora*. The posterior extension of the posterior sorting area is developed as a blind

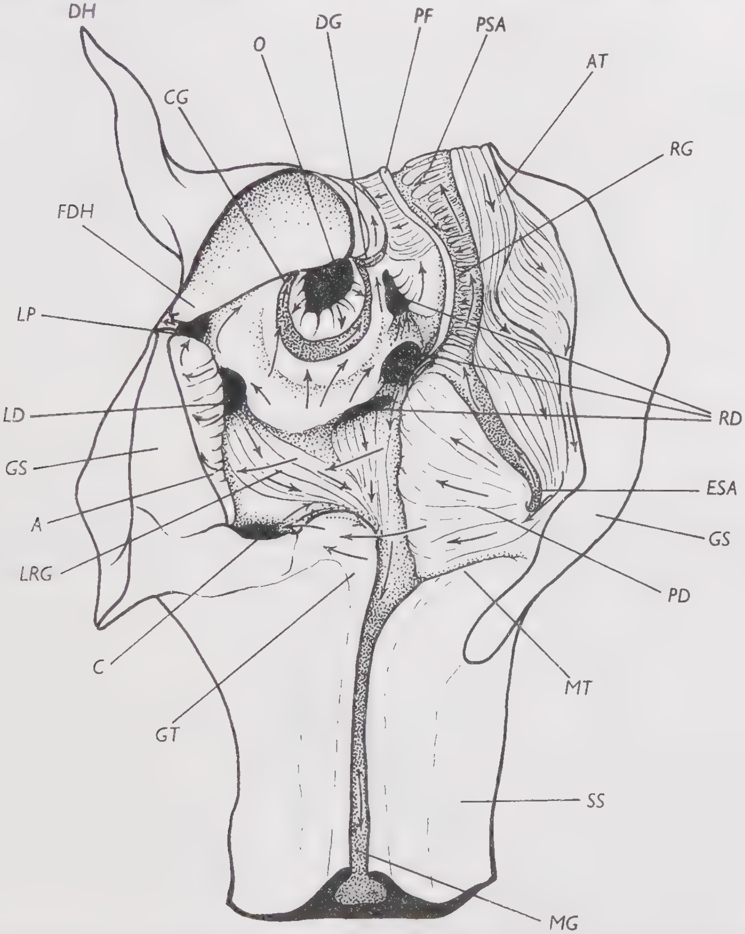

Fig. 8. The stomach dissected by a longitudinal cut through the centre of the gastric shield. The dorsal hood has not been opened. The direction of the ciliary currents are shown. *A*, collecting area; *AT*, acceptance tract; *C*, caecum; *CG*, circular groove; *DG*, dorsal groove; *DH*, dorsal hood; *ESA*, extension of the posterior sorting area; *FDH*, dorsal hood flange of the gastric shield; *GS*, gastric shield; *GT*, major typhlosole; *LD*, isolated left aperture; *LP*, left pouch; *LRG*, lateral rejection grooves; *MG*, mid-gut; *MT*, minor typhlosole; *PD*, pad; *PF*, posterior fold; *PSA*, posterior sorting area; *RD*, isolated right ducts; *RG*, rejection groove. Other lettering as before.

pocket. The walls of this extension are without ridges and all particles on them are carried forward to the posterior sorting area. The extension is adjacent to a constriction in the acceptance tract where the latter fans out as a pad of tissue, described above. It is probable that this constriction controls the size of the particles passing on to the pad, and so to the apertures of the digestive diverticula. Particles wider than the constriction contact the strongly beating

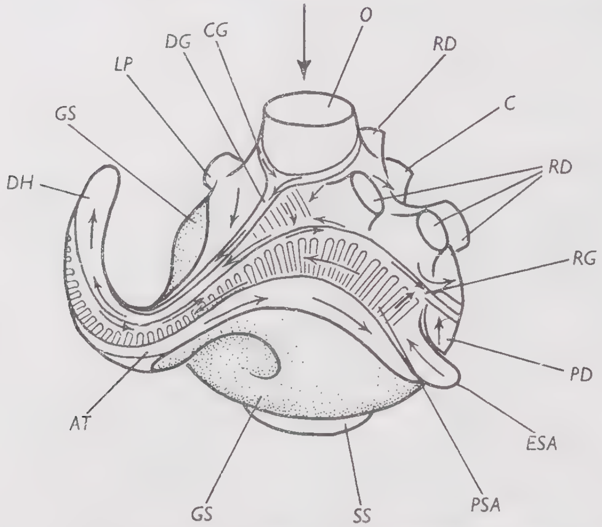

Fig. 9. The head of the stomach, drawn from a model constructed from details obtained by dissection and serial sections. The outline of the various structures and sorting areas together with the direction of the ciliary currents were drawn on the outside of the model. Lettering as before.

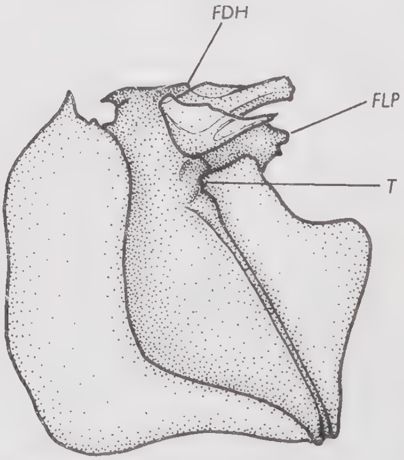

Fig. 10. Drawing of the gastric shield removed from the stomach. *FLP*, left pouch flange of the gastric shield; *T*, tooth. Other lettering as before.

cilia of the extension and are diverted to the posterior sorting area. The replacement of an intestinal groove by a series of grooves (lateral rejection grooves) as was first described in *Pandora*, also occurs in *Cochlodesma*. The lateral rejection grooves extend from the apertures of the digestive diverticula to the mid-gut opening. In the depths of the grooves rejected material from the diverticula is carried to the mid-gut, while accepted material circulating in the stomach is carried across the grooves by the action of the cilia of the crests between the grooves.

The extent of the major typhlosole in *Cochlodesma* is also similar to that in *Pandora*. The typhlosole terminates in the entrance of what is probably the right caecum. In the description of *Pandora* (Allen, 1954) the possibility that the opening immediately posterior to the left pouch (present in *Cochlodesma*) is homologous to the left caecum is discussed. While the arguments still hold in the case of *Cochlodesma*, Purchon, in a personal communication, has suggested that there is possibly only the one caecum and that the opening on the left side, posterior to the left pouch, is comparable to the isolated ducts of the right side—even though several ducts open at this one aperture.

Apart from these divergences the basic pattern of circulation of particles in the stomach is as described by Owen (1953, 1955) for other Eulamellibranchia.

Digestive diverticula

The organization of the digestive diverticula is similar to that of other lamellibranchs (Yonge, 1926; Owen, 1955). They form a compact organ anterior to the stomach (Fig. 6) composed of main and secondary ducts and tubules. The main ducts are short and similar in cross-section to those of *Zirphaea crispata* (Owen, 1955) (Fig. 11 A). The lumen is deeply grooved, the number of grooves varying from four to seven. A single ciliated tract is present. Main and secondary ducts do not differ histologically from those of other Eulamellibranchia (Owen, 1955).

The digestive tubules, clustered at the distal end of each secondary duct, are elongate (Fig. 11 B). They are unusual in that there are a number of minor branches along the length of each tubule. Each minor branch terminates at a single, large cell filled with a clear fluid. Non-ciliated crypt cells are situated close to this large cell and the remainder of the tubule is lined with typical vacuolated cells. Similar large cells have been reported in *Lasaea rubra* by Oldfield (1955) and Morton (1956) and in the Lucinacea (Allen, in the press). It is possible that, if the suggestion (Owen, 1955) that fluid is drawn into the tubule by the absorptive function of the cells is correct, these large cells carry out the major part of the absorption. Their terminal position and large size, almost entirely filled with a single vacuole containing a fluid and no particulate matter, suggests that this may well be the case, digestive function being confined to the vacuolated cells.

SIZE AND AGE GROUPS

On three occasions *Cochlodesma* was obtained in sufficient numbers to give information on the age and size grouping of the Newton population. Shell markings give no indication of age and annual growth rate. The sieve used (aperture size 3 mm^2) did not retain shells less than 5 mm long, and examination of material not retained by it showed that one size-group had passed through in each of the samples. The approximate average length of this group

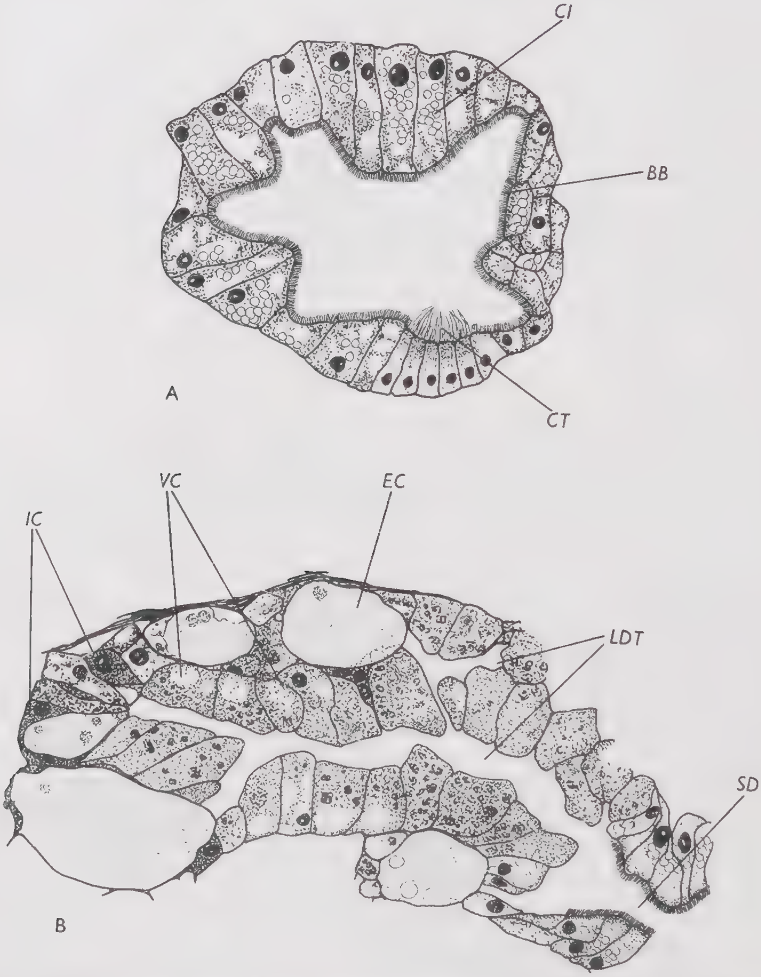

Fig. 11. A. Transverse section of a main duct of the digestive diverticula. *BB*, brush border; *CI*, refractive cell inclusions; *CT*, ciliated tract. B. Longitudinal section of a tubule to show the minor branching. *EC*, large terminal cell; *IC*, non-ciliated crypt cells; *LDT*, lumen of the digestive tubule; *SD*, secondary duct; *VC*, vacuolated cell.

is indicated by arrows in Fig. 12. The figure shows length/number plots of the three samples. The Newton population comprises four or possibly five size groups. Regular sampling showed that mature sperm and eggs are present only during early November. Groups one and two are immature. Thus, the size groups are almost certainly year groups, *Cochlodesma* not living more than 5 years—the majority for only 4—on this beach. There is a fairly steady increase in length of about 4 mm per year during the life of the animal.

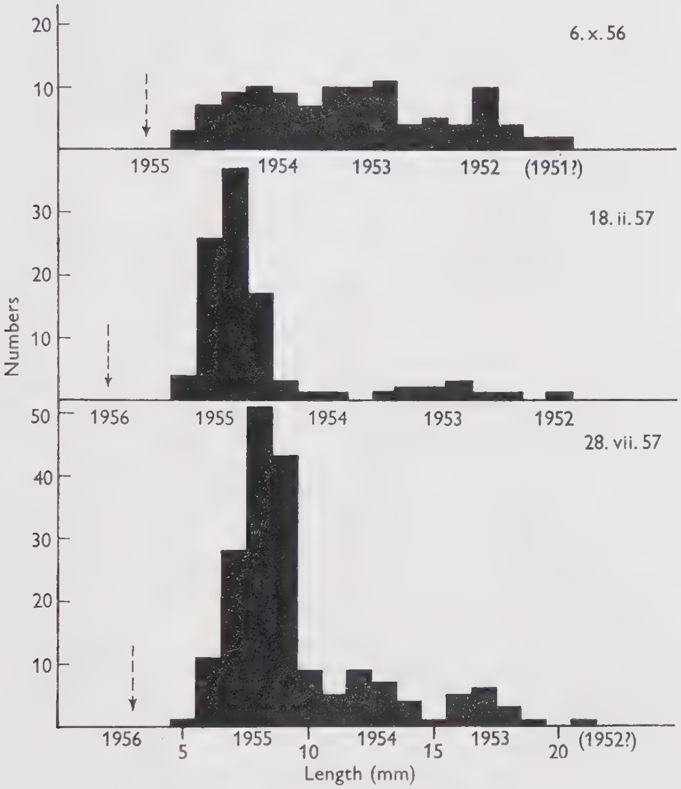

Fig. 12. Histograms of the length/number relationship of samples from the Newton population of *Cochlodesma praetenue*.

The longest shell recorded was 21 mm. This is by no means large in comparison with shells from the west coast of Scotland. Prof. A. D. Hobson has kindly given me a number of specimens which he collected from the Island of Muck which are between 30 and 32 mm in length. Forbes & Hanley (1853) record even larger specimens from deep water off Aberdeen. The specimens from the Island of Muck give no indication whether their growth rate is faster, or whether they live longer than the Northumberland specimens.

DISCUSSION

The Anomalodesmata are rarely found in great numbers and must be regarded as being amongst the least successful of Lamellibranchia inhabiting sands and muds. Yonge (1952) emphasized this in his studies on the Lyonsiidae, and showed that in this family there was adaptation for sedentary life. A similar, but less marked tendency toward sedentary life is seen in the related families, Thraciidae, Pandoridae and Laternulidae. There is no byssal attachment, and therefore these animals are usually found in sheltered conditions. In particular this was shown to be the case in *Pandora* where the extremely inequivalve shell is probably an adaptation to a sedentary life on or close to the surface of the substratum (Allen, 1954; Allen & Allen, 1955). Although buried, the somewhat inequivalve *Cochlodesma*, like *Pandora*, adopts a horizontal position. The formation by *Cochlodesma* and *Thracia* of mucus-lined inhalant and exhalant tubes also must be related to a sedentary existence, as the animal must remain stationary for such fragile tubes to function. The siphons need no longer extend to the surface and suffer from the attacks of other animals. As there is no attachment by a byssus there is no development of the heteromyarian condition, such as is seen in the Lyonsiidae (Yonge, 1952).

The union of the valves dorsally by the periostracum is probably a primitive feature. Yonge (1952) correlates this with a lack of hinge teeth and this is no doubt true of *Cochlodesma*. In addition, this lack of hinge teeth is probably correlated with the complexity of the ligament.

Consideration of these features leads to the belief that the Anomalodesmata have had a long, separate evolutionary history from that of other eulamellibranchs. This belief is supported by differences in the morphology of the stomach, found both in *Pandora* and *Cochlodesma*, from that in other Eulamellibranchia. These differences are notably the replacement of the intestinal groove by the lateral rejection grooves and the extent of the major typhlosole. The homologies of the caecum and of an opening on the left side of the stomach posterior to the left pouch are not clear. The form of the ctenidium (Atkins, 1937*b*) with its reduced and reflected outer demibranch again is characteristic of the suborder.

Cochlodesma is highly specialized for a sedentary life in sheltered conditions at low-water mark and below. Although it is closely related to the Thraciidae, Pandoridae and Lyonsiidae it possesses characteristics that clearly distinguish it from these related forms.

SUMMARY

Cochlodesma praetenue is well adapted for a sedentary life in sheltered conditions in soft substrata at low-water mark to a depth of 60 fathoms.

The habits, morphology and ciliary feeding and cleansing mechanisms are described and compared with those of members of the related families Thraciidae, Pandoridae and Lyonsiidae.

The complex ligament is described. The mantle edges are extensively fused. The detailed structure of the siphons is described and related to their function of laying down mucus-lined inhalant and exhalant tubes.

The morphology and ciliary currents of the stomach show modifications similar to those previously described for *Pandora*. These modifications involve the extent of the major typhlosole and the replacement of an intestinal groove by lateral rejection grooves.

Analysis of three large samples shows that *Cochlodesma* from the Northumberland coast live for a maximum of 5 years. These animals become mature in their third year, spawning in early November.

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EUDACTYLINA RACHELAE N.SP., A COPEPOD PARASITIC ON THE ELECTRIC RAY, *TORPEDO NOBILIANA* BONAPARTE

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(Text-fig. 1)

Five species of the genus *Eudactylina* are known from British waters (Scott & Scott, 1913), and of these only one, *E. acuta* Van Beneden, is recorded in the *Plymouth Marine Fauna* (Marine Biological Association, 1957, p. 181). The species described below was found among the gill filaments of a large specimen of *Torpedo nobiliana* Bonaparte (det. P. G. Corbin) taken in trawl by R.V. *Sula* off Plymouth in July 1957. There were five females.

Eudactylina rachelae n.sp.

Description of the female

The body is elongated, maggot-shaped. The length, excluding the egg strings, is 2·3–2·5 mm. When fresh the colour is cream, with the gut showing through brown. The first pedigerous segment is fused with the head, the whole being covered by a single dorsal shield which is one and a third times as long as wide, and somewhat narrowed anteriorly. The second and third pedigerous segments have dorsal shields which are similar in length, while that of the fourth pedigerous segment is longer. The dorsal shield of the fifth pedigerous segment is smaller than those of the second and third. The genital segment is narrower than the preceding segments. The urosome (excluding the genital segment) has two segments; the first being roughly twice as long as the second. The dorsal surface of the body bears numerous crescent-shaped lamellar processes, which look like minute spinules when viewed from the side.

The antennules (Fig. 1 B) are stout, of five podomeres, the last indistinctly separated from the fourth. The armature is similar to that of *E. acuta* and *E. similis*. The two principal spines lack the fringe of minute spinules along the upper edge, which is found in *E. similis*. The large spine on the second podomere has a series of minute crescent-shaped depressions, each of which appears to contain a spinule lying almost parallel to the border of the spine. The large spine on the third podomere has three shallow oblique teeth on each side (Fig. 1 C).

The antennae (Fig. 1 D) have four podomeres. The second podomere has a strong spine-like process on the inner side, while the third has two such processes and a seta. The third podomere also bears numerous crescent-shaped processes similar to those on the dorsal surface of the body. The

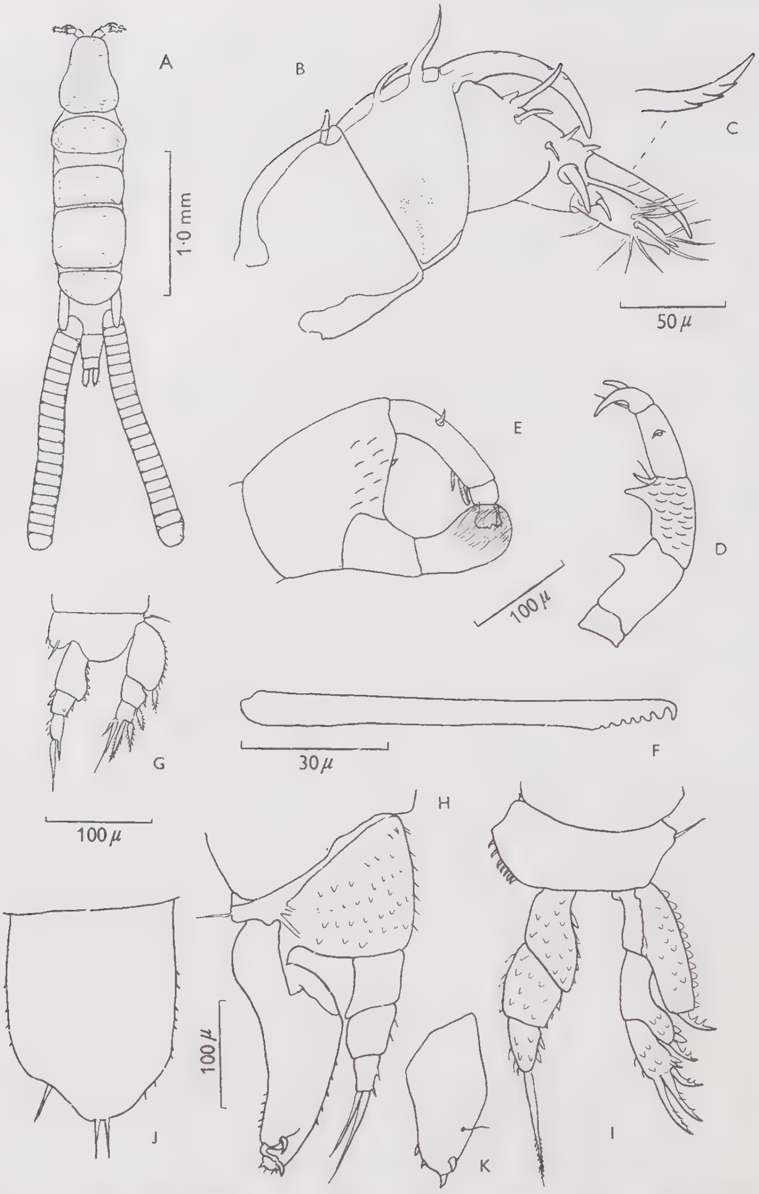
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Fig. 1. A–K, *Eudactylina rachelae* n.sp., adult female. A, dorsal view; B, antennule; C, spine on third podomere of antennule; D, antenna; E, second maxilliped; F, mandible; G, first leg; H, second leg; I, third leg; J, fifth leg; K, ventral view of left caudal ramus. G–K are all drawn to the same scale.

fourth podomere bears what appears to be a sensory pit. The antenna ends in two stout spines and a seta.

The mandible (Fig. 1 F) is long and straight, with a terminal hook and seven or eight short blunt teeth.

The maxilla, like that of *E. similis*, has two setae on the principal lobe and one on the secondary lobe.

The first maxilliped has three podomeres and terminates in a stout spine with two tufts of setae at its base.

The second maxillipeds (Fig. 1 E) are large and chelate. The movable branch of the chela ends in an expanded cup-like structure which fits inside the thin, expanded and minutely fluted end of the immovable branch.

The first pair of legs is much smaller than the second to fourth pairs. The second basal podomere has one moderate and two small spines on its inner border. The endopod and exopod each have three podomeres.

The second legs have strongly modified exopods (Fig. 1 H), while the endopod has three normal podomeres.

The third and fourth legs are similar in structure (Fig. 1 I). The surface of the podomeres is drawn out into flattened spine-like processes which appear as short triangular lamellae when viewed from the side. The strong spines on the exopods are distinctly toothed.

The fifth leg consists of a single plate-like podomere, with three terminal setae and a few spinules along the lateral borders.

The caudal rami are one and a half times as long as the anal segment. Each ramus has two short terminal spines, two weaker inner spines and one feeble outer seta.

Remarks

This species is closely related to *E. similis* T. Scott, but can be separated by the differences given in Table 1.

TABLE 1

E. similis T. Scott	*E. rachelae* n.sp.
Large spine on third podomere of antennule with minute spinules along upper border	Minute spinules not present on this spine, but three oblique teeth present on each side
Mandible curved	Mandible straight
Movable branch of second maxilliped ends in a spine	Movable branch of second maxilliped ends in an expanded cup
First legs not much smaller than others. The inner border of the second basal podomere with two stout spines	First legs much smaller than others. Inner border of the second basal podomere with one moderate and two small spines
Spines on third and fourth legs not toothed	Spines on third and fourth legs toothed
Caudal rami without two inner spines	Caudal rami with two inner spines

Type material. The holotype and one paratype will be deposited at the British Museum. Three paratypes will be retained in my collection.

These specimens were collected while working at the Plymouth Marine Laboratory; my thanks are due to the Director and Staff for the facilities provided. My thanks are also due to Dr J. Llewellyn for bringing these copepods to my notice.

SUMMARY

Eudactylina rachelae n.sp. is described and figured. This copepod was found among the gill filaments of *Torpedo nobiliana* Bonaparte caught off Plymouth in July 1957.

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THE LOCALIZATION OF ORGANICALLY BOUND IODINE IN THE ENDOSTYLE OF *AMPHIOXUS*

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(Plate I and Text-fig. 1)

Recent work has done much to strengthen, from a functional aspect, the classical view that the thyroid gland of vertebrates is homologous with the endostyle of the protochordates (Barrington & Franchi, 1956*a*), for the presence of organically bound iodine has been detected in the latter organ, both in *Ciona* and in other ascidians (Barrington, 1957 and unpublished work) and in *Amphioxus* (Thomas, 1956). The occurrence of iodine binding in the endostyle of the ammocoete larva of the lamprey (Gorbman & Creaser, 1942) is now known to be associated with a secretion which in certain properties resembles thyroid colloid, and may well be homologous with it (Barrington & Franchi, 1956*b*). The presence of a similar secretion in the *Ciona* endostyle (Barrington, 1957) implies that a specialized thyroidal biosynthesis is also operative there, and that the iodine binding is functionally significant for the organism and is not a biochemical 'accident' as the binding which takes place in the scleroproteins of many invertebrates has been thought to be (Gorbman, 1955).

It is thus a matter of importance to determine the cytological basis for the iodination which occurs in the endostyle of *Amphioxus*. According to Thomas (1956) the dorsal glandular tract is associated with this process, a situation quite different from that found either in the ammocoete larva or *Ciona*, and it is the purpose of the present work to examine this matter in detail as a basis for a comparison of the functional organization of the three types of endostyle.

MATERIAL AND METHODS

The work was carried out in part at the Plymouth Laboratory of the Marine Biological Association, and in part at Nottingham, the methods being substantially similar to those used in previous work on the ammocoete and *Ciona* referred to above. For radioactive studies the animals were immersed at 10° C in sea water containing 200 μc of ^{131}I per litre; this is one-tenth of the concentration used by Thomas (1956), who also immersed the animals for 15–17 h, whereas at Nottingham a period of 48 h has been adopted as a standard in all such experiments. Specimens were mostly fixed in Susa and in

Bouin's fluid in sea water, and autoradiographs were prepared by the stripping-film technique of Pelc (Pearse, 1954).

The endostyle is commonly described as a mucus-secreting organ, but the term 'mucus' is used so loosely in much zoological literature, to mean simply a viscous secretion, that it has been essential in the present work to select staining and histochemical methods which would give a more precise definition to it. The PAS reaction (counterstained with Harris's haematoxylin, followed either by metanil yellow or by tartrazine) has therefore been used (controlled by acetylation) to demonstrate the presence of 1:2-glycol groups, and toluidine blue metachromasia and the alcian blue method of Steedman (1950) to show whether or not the reacting secretion contains acid mucopolysaccharides, the importance of the latter being that they appear to be the main components of epithelial mucins (Pearse, 1954). The selectivity of the alcian blue was found to be much improved by the addition of an equal volume of 1% acetic acid to the 1% solution of the dye, as suggested by Lison (1954). The classical mucicarmine technique of Mayer, using Southgate's method of preparing the staining solution, has also been valuable, as has the Azan procedure.

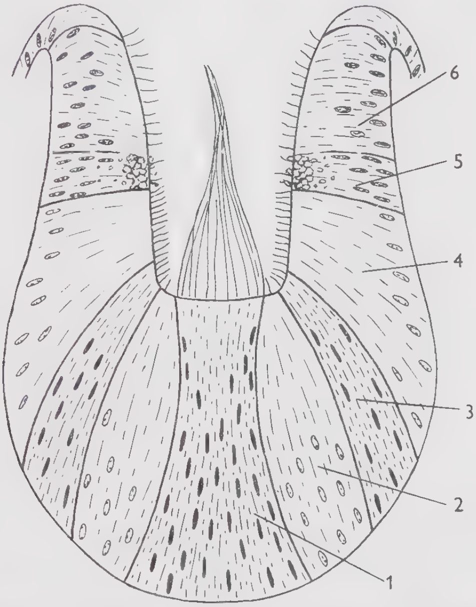
There has been no serious difficulty in interpreting the results of these tests, although there has been a good deal of quantitative variation; animals, for example, which have been fixed and processed side by side, may show considerable differences in the intensity of their response to mucicarmine, even in such clearly defined cell types as the secretory cells of the gill bars. It seems probable that although on superficial inspection *Amphioxus* seems to survive well under laboratory conditions, there is, in fact, much variation in the physiological condition of different specimens by the time that they are fixed. In confirmation of this, it is of interest to note that Možejko (1913) concluded that variation in the extent to which the tissues of living *Amphioxus* were coloured by ingested carmine reflected differences in their physiological condition. It is also clear that another source of variation arises from the fact that the secretory contents of the cells, and sometimes even cytoplasm and nuclei, may be discharged from the endostylar epithelium under the stress of fixation. The present account has therefore been based on examination of a number of specimens fixed at different times under different conditions, some at Plymouth and some at Nottingham, and there is no reason to suppose that the conclusions are in any way affected by such variations.

OBSERVATIONS

Organization of the endostyle

Previous workers have entirely overlooked what, from the point of view of the interpretation of the iodination process, is the most important feature of the endostyle, and it is therefore necessary to give a brief new description of its organization (Text-fig. 1; Pl. I, fig. 1).

At the base of the endostylar groove is a group of cells, here termed 'zone 1', which closely resemble the correspondingly placed zone 1 cells of *Ciona* (Barrington, 1957). They possess slender and densely staining nuclei, long cilia, and a conspicuously striated cell border. This ciliation and border are characteristic of the whole of the endostylar epithelium, although their degrees of development vary greatly from zone to zone. Secretory droplets are present in zone 1, and are strongly PAS-positive, the general cytoplasm also giving a positive reaction. These droplets stain with mucicarmine, and the fact that they stain selectively with alcian blue, and that toluidine blue gives a definite gamma (red) metachromasia in the cells, indicates the presence of acid mucopolysaccharides. It may thus be concluded that these cells are producing an epithelial mucin, in the sense in which the term has been used above; this secretion is particularly dense at the lateral edges of the zone (Pl. I, fig. 4), from which it is discharged over the surface of the mass of long cilia, a situation which is in line with Orton's (1913) suggestion that the main function of these cilia is to deflect the endostylar secretion so that it passes out on to the lateral walls of the pharynx.



Text-fig. 1. Diagrammatic transverse section across the endostylar epithelium of *Amphioxus* to show the distribution of the several zones, which are indicated by corresponding numbers. For further explanation see text.

On either side of zone 1 lie the ventral glandular tracts (zone 2). These bear only a few cilia, while the nuclei are less densely stained than those of zone 1 and have a prominent nucleolus. PAS-positive droplets are present, and these react positively also with mucicarmine and alcian blue, while the cells show gamma metachromasia. The reactions are thus similar to those of zone 1, but they are usually less intense; it seems likely, then, that the secretion of these tracts contains acid mucopolysaccharides but that it may well differ from the secretion produced by zone 1.

Zone 3 is a narrow zone separating the ventral glandular tracts from the dorsal ones. The nuclei are much as in zone 1, but the cilia are considerably shorter, although very densely developed. The distal cytoplasm has a somewhat vacuolated appearance, and slight positive responses to the various histochemical reagents indicate some degree of secretory capacity and, probably, the presence of acid mucopolysaccharides.

The dorsal glandular tracts (zone 4) have nuclei and ciliation substantially similar to those of the ventral tract, but in other respects the two tracts are quite different, as is true of the corresponding tracts in *Ciona* (Barrington, 1957) and the ammocoete (Barrington & Franchi, 1956*b*). This difference is apparent even in Azan preparations, in which the ventral tract cells show an indefinite blue flocculation while those of the dorsal tract have an orange-stained cytoplasm with faintly blue-stained droplets. These droplets are PAS-positive, although less strongly so than those of the ventral tracts, but a clear difference from the latter is shown in the negative response of the cells to mucicarmine and to the tests for acid mucopolysaccharides (Pl. I, fig. 4).

Zone 5 is a group of cells lying immediately above the dorsal glandular tracts. The nuclei here are densely granular but less intensely stained and less compressed than in zones 1 and 3, and the ciliation, while detectable, is exceedingly sparse. The important characteristic of these cells is their clearly defined secretion (Pl. I, figs. 1, 4, and diagrammatically in Text-fig. 1). This can be seen as very fine droplets in the cytoplasm, sometimes extending down to the base of the cell, but is particularly conspicuous as an accumulation in the more distal parts, where it extends through the cell border (the striations of which are less clearly defined than elsewhere in the endostyle) and on to the occasional cilia. The extrusion of fine threads of this secretion is often evident, and there is much variation from animal to animal in the amount of secretion visible in the cell, but this is probably influenced by the factors mentioned earlier (p. 118). Such variation may, however, account for this particular region not having been previously described, for in well-fixed specimens the accumulation of secretion in these cells is impossible to overlook and, indeed, it provides the most conspicuous stainable feature of the whole endostyle. This secretion, which stains well with aniline blue in the Azan procedure, contrasts sharply with the product of the adjacent dorsal glandular tracts, for it is very strongly PAS-positive, and also reacts positively with mucicarmine and alcian blue, and shows gamma metachromasia with toluidine blue. The only other region of the endostyle which is comparable with it in the clarity of these reactions is the lateral edge of zone 1, and even so the reactions of zone 5 tend to be more brilliant.

Zone 6 differs from zone 5 in the much greater density of ciliation and the greater chromophilia of the nuclei. Very small PAS-positive droplets may be visible distally and may indicate, as in zone 3, a rudimentary secretory capacity. Zone 5, in fact, has the appearance of having been differentiated out of zone 6 by a development of its secretory capacity and a reduction of its ciliation.

The above observations lead to the conclusion that zones 1 and 5 are the parts of the endostyle which are most obviously mucin-secreting in the sense of producing a secretion which is rich in acid mucopolysaccharides. The latter appear also to be present in the ventral glandular tracts, and probably in zone 3, but are certainly absent from the secretion of the dorsal glandular tracts. Here, then, as in *Ciona* and the ammocoete, it is evident that the endostyle is far from being a simple 'mucus-secreting' organ, and that much remains to be learned as to the nature and functions of its secretions.

Autoradiographs

Transverse sections (Pl. I, fig. 2) clearly show that bound iodine is restricted in the endostyle to two areas, lying one on each side immediately above the dorsal ciliated tracts, and that these areas correspond precisely to the groups of cells described above as zone 5.

Each area extends over the upper ends of the cells and also into the endostylar lumen (Pl. I, figs. 3, 5). One must assume that random scatter contributes to the peripheral part of the image, but the central point of the latter can be taken as the centre of distribution of the iodine, and this appears to be located, as far as one can judge, at about the level of the surface of the epithelium (Pl. I, figs. 4, 5, and also fig. 3). In other words, it lies somewhat distal to the centre of the area which is commonly occupied by the stored secretory contents of these cells. If, then, one assumes, as seems reasonable, that it is these contents which become iodinated, it seems likely that the iodination process takes place at or very near to the cell border. The autograph image may thus represent an iodinated secretion which, at least in part, has probably been discharged from the cells and which is bound to their surface and, perhaps, to their cilia. Some secretion in process of being discharged is often visible in sections, but it is not always present, and by no means corresponds exactly in amount or shape to the iodine image. Such material, in fact, need not necessarily be iodinated, for, as mentioned above, its discharge is probably in part a consequence of contraction during fixation, and the image may well be produced largely by a substance which is not actually visible in stained sections. A precisely similar situation obtains in the endostyle of the ammocoete larva (Barrington & Franchi, 1956*b*), where the secretion is difficult to identify once it has left the cells, and where a clearly defined iodine image overlies cilia which have little, if any, visible secretion associated with them.

It follows from the above that the iodinated secretion is normally discharged into the lumen of the pharynx, and this is confirmed by the existence of radio-iodine images in the lumen adjacent to the endostylar cells. Such are also to be found in the food cord in the dorsal groove, into which, as is well known, material secreted by the endostyle and gill bars is swept in consequence of the action of the pharyngeal ciliation, while the food cords in the mid-gut and intestine also give strong images. It is always to be remembered that food organisms themselves may bind iodine and contribute to such images (Barrington, 1957), but it seems wholly improbable that this could account for all of the latter; I agree, therefore, with Thomas (1956), who noted similar images in his own preparations, that the iodinated secretion of the endostyle is ultimately incorporated into the food cords.

Thomas noted that the amount of iodine taken up by *Amphioxus* increased to reach a maximum in about 7–10 days after they were caught. My own preparations were obtained from animals of which some were fixed at Plymouth within 5 days of being caught, while others were fixed at Nottingham, 3–4 weeks after being brought into the Plymouth Laboratory. All gave essentially similar autoradiographs. In none of them was there any certain sign of organically-bound iodine in any other part of the endostyle, such slight spots as were visible being indistinguishable from random scatter and background fogging. The absence of iodine from the lateral edges of zone 1, where there are usually distinctive masses of secretion which are histochemically similar to those of the thyroidal cells, is particularly striking (Pl. I, figs. 3, 5). One is driven to conclude, then, that iodine binding in this endostyle, as in that of *Ciona*, is not a generalized or random process, but is a specialized property of a specific group of cells.

DISCUSSION

The facts described here bring the iodine-binding activities of the endostyle of *Amphioxus* into closer line with those of *Ciona* and the ammocoete than was apparent from Thomas's account. He described the images as being 'situated near the periphery of and slightly lateral to the lateral series of mucous glands', with which I would agree, although I should not myself use the term 'mucous' (see below). Further on, however, he says that 'the most obvious centre of radioactivity in ^{131}I-treated animals is in the mucus of the lateral mucous glands'. Of this my own preparations give no evidence. On the contrary, the present account defines the iodine binding as a property of a specialized group of mucus-secreting cells lying immediately above (lateral to) the lateral glandular tracts, and it is important to bear in mind that these tracts, the lateral mucous glands of Thomas's description, do not secrete mucus at all in the sense of a substance rich in acid mucopolysaccharides.

This localization of function agrees very well with the situation in *Ciona*; in that animal there is, as compared with *Amphioxus*, an additional glandular tract on each side, but the region where iodination occurs lies immediately above the glandular tracts in a position which essentially corresponds with that of the iodination centre in *Amphioxus*. It is doubtful whether any significant iodination occurs in the tracts of *Ciona*, and it is certain that none does so in those of the ammocoete. Iodination extends over a much wider area of the endostylar epithelium of the latter than it does in the protochordates, but the regions chiefly concerned bear essentially the same relationship to the glandular tracts as do the iodination centres in *Amphioxus* and *Ciona*, allowing, of course, for the much greater complexity of the organ in the ammocoete.

In view of this fundamental morphological similarity, it is all the more interesting to find from the present work that there is a significant difference in certain histochemical properties of the iodinated material. Evidence has already been given that in the ammocoete larva (Barrington & Franchi, 1956*b*) this material resembles thyroid colloid in showing, as far as these histochemical procedures go, the characteristic properties of a glycoprotein (or mucoprotein, since the two are not distinguishable by these methods). Renewed tests, in conjunction with the present work, have confirmed that this ammocoete secretion, like mammalian thyroid colloid, is negative to mucicarmine and alcian blue, and that it does not exhibit gamma metachromasia, and it may be added that the same appears to be true of *Ciona*, although the ascidians need much more extended study before this statement can be regarded as sufficiently broadly grounded. In *Amphioxus*, on the contrary, the secretion includes an acid mucopolysaccharide component, and in this respect resembles typical epithelial mucins, including those of certain other parts of the endostyle and of parts of the gill bars and dorsal groove of the pharynx. This appears, in fact, to be the first reported case of the association of iodine binding with an epithelial mucin, characterized as such by its localization and its histochemical properties.

In this connexion Thomas (1956) has suggested from his own work on *Amphioxus* that thyroid colloid 'is the direct evolutionary successor to the endostylar mucus of a protochordate ancestor'. The present results confirm and clarify this suggestion, but it is very important to appreciate that they show that iodine-binding in *Amphioxus* is not a generalized binding to all endostylar mucus. None is associated with the secretion of zone 1, the acid mucopolysaccharide properties of which are indistinguishable from those of the thyroidal region of zone 5, and this means that in *Amphioxus*, just as in *Ciona* (Barrington, 1957), iodine-binding is a specialized biochemical property of a particular group of cells. This conclusion carries the implication that the product is not the result of random iodination, but that it is of biochemical significance to the organism. As Thomas (1956) points out, the work of

Sembrat (1953) gives some reason for supposing that this product can be regarded as resembling thyroid hormone. It seems quite possible, then, from the situation in *Amphioxus*, as it does from that in *Ciona* (Barrington, 1957), that the production of thyroid hormone may already be established at the protochordate level of chordate evolution but that it is passed into the lumen of the pharynx instead of being secreted directly into the blood stream. It may well be, as suggested by Thomas's work, that it is then taken up into the body through the epithelium of the alimentary canal as part of the normal process of digestion and absorption of food, but it is hoped to report further on this aspect in a later publication.

I am much indebted to the Director and Staff of the Plymouth Laboratory for the trouble taken to provide me with the animals and facilities needed for this work, to Mr T. Berbank for taking the photomicrographs, and to Miss J. Plumtree for technical assistance.

SUMMARY

An account is given of some features of the organization of the endostyle of *Amphioxus*, with particular reference to the distribution of sites of mucus secretion as indicated by positive responses to tests for acid mucopolysaccharides. Autoradiography shows that the centre of distribution of organically-bound iodine is associated with the tips of the mucus-secreting cells of zone 5, and not with the glandular tracts. Mucus secretion also occurs elsewhere in the endostyle (although not in the dorsal glandular tracts), but as it is not associated with any accumulation of bound iodine it is concluded that the binding in zone 5 is a specialized property of the cells of that particular zone. The results are discussed in the light of recent work on the protochordates and the ammocoete larva, and it is concluded that in *Amphioxus*, as in the Tunicata, there is reason for supposing that the iodination process is a biochemical specialization and that its product must therefore be of some physiological significance to the organism. Attention is also drawn to the evolutionary interest of the association of iodine-binding with a mucin rather than with a glycoprotein.

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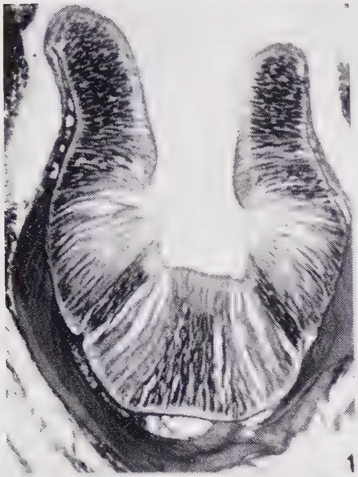

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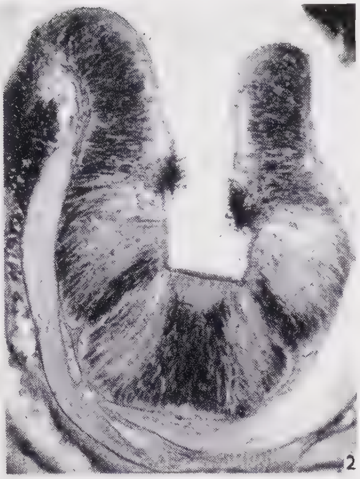

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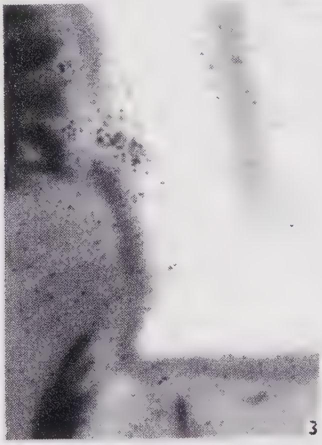

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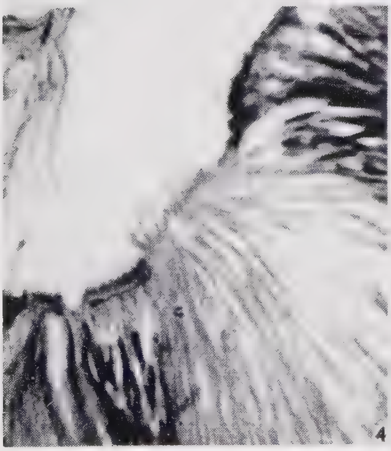

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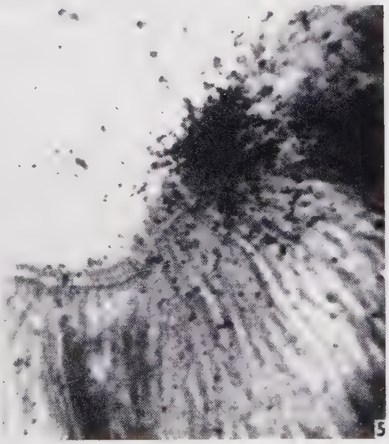

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EXPLANATION OF PLATE I

Fig. 1. Transverse section of the endostyle of *Amphioxus*, which should be compared with Text-fig. 1 for the identification of the several zones. Zones 2 and 4 (ventral and dorsal glandular tracts) can be distinguished by the lighter staining and more basal location of their nuclei. The PAS-positive secretion (slightly darker) at the distal ends of the zone 5 cells is clearly visible on the right side of the endostyle at the point where the epithelium swells slightly to the left. PAS, Harris's haematoxylin and tartrazine.

Fig. 2. Autoradiograph of a section adjacent to that of fig. 1. The black iodine image is centred over the tips of the zone 5 cells.

Fig. 3. Autoradiograph of a transverse section of the left side of an endostyle. Zone 3, with dark nuclei, lies at the right-angled bend; zones 2 and 1 lie to its right, while zone 4 (dorsal glandular tract) lies above it. Zone 5 lies immediately above zone 4 and continues into zone 6. An unusually light iodine image has been obtained as a result of the use of a slightly over-age stripping-film, and is clearly seen to extend into the lumen from the tips of the zone 5 cells; the small amount of secretion accumulated at the distal ends of these is represented by a negative image. Note the absence of any iodine image over the mucus-secreting cells of zone 1, which lie at the bottom right-hand corner; the background of scattered spots is a result of fogging.

Fig. 4. Transverse section of the right side of an endostyle, stained with alcian blue (Harris's haematoxylin as counterstain). Zone 4 (dorsal glandular tract) is the light area occupying the middle of the region photographed, and is completely negative. A strong positive response is seen in the distal ends of the zone 5 cells, lying immediately above zone 4; a weaker reaction is just detectable in zones 3 and 2, but a strong one is shown at the right edge of zone 1, which just comes into view at the extreme left margin.

Fig. 5. Autoradiograph of a section adjacent to that of Fig. 4. The centre of the dense black iodine image overlies the tips of the zone 5 cells, the position of which can be gauged by comparison with Fig. 4. Note the complete absence of iodine accumulation elsewhere, even over the edge of zone 1, despite the positive alcian blue (mucin) response which this shows in Fig. 4.

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THE CHEMISTRY OF ETHYLENEDIAMINE TETRA-ACETIC ACID IN SEA WATER

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(Text-figs. 1–10)

In recent years, ethylenediamine tetra-acetic acid (EDTA) has been extensively used in culture media for unicellular algae. This, and other chelating agents are also becoming widely used in toxicity studies with plants and animals, in analysis and for controlling the ionic concentration of certain metal ions in a wide variety of studies.

EDTA forms complexes with a large number of metallic cations, and in the presence of sufficient EDTA only very small amounts of many metals can exist as free ions. The use of EDTA as a metal buffer system has been advocated by numerous workers. These metal buffer systems depend for their operation upon the equilibrium between the metal cations, the EDTA and the chelate. Providing that the concentrations of the chelate and the chelating ion are large compared with that of the free metal ion, such a system will tend to maintain a constant concentration of metallic ions, should these be continuously removed by a biological system.

The available data suggest that EDTA is not readily metabolized by most forms of life, and moreover it does not appear to be markedly toxic except by virtue of its metal-chelating reactions. Its use under the correct conditions for controlling metal ion concentrations in biological systems therefore has much to commend it.

The physical chemistry of metal–EDTA equilibria has been examined by several workers (Chaberek, Bersworth & Martell, 1955; Raaflaub, 1956) and methods for calculating the concentrations of free metal ion at equilibrium outlined. These treatments, however, are limited to simple cases when only one chelating metal ion is present. The biologist is inevitably faced with working with systems containing many reacting metal ions (e.g. in sea water). Data regarding the free metal ion concentrations existing in equilibrium with EDTA in such systems is of obvious importance if the use of EDTA is to be fully exploited. This paper records some data on the equilibria set up when EDTA is added to sea water and on the effect of variations of certain controlling factors.

THE DISSOCIATION OF EDTA

In distilled water EDTA behaves as a typical quadribasic weak acid.

$$H_4Y \rightleftharpoons H_3Y^{-1} + H^+$$

$$H_3Y^{-1} \rightleftharpoons H_2Y^{-2} + H^+$$

$$H_2Y^{-2} \rightleftharpoons HY^{-3} + H^+$$

$$HY^{-3} \rightleftharpoons Y^{-4} + H^+$$

where Y^{-4} represents the fully dissociated EDTA quadrivalent anion. The third and fourth dissociation constants may be determined directly from the titration curve which gives values of $pK_3 = 6{\cdot}1$ and $pK_4 = 10{\cdot}2$. The first and second dissociation constants must be determined indirectly and the reported values are $pK_1 = 1{\cdot}9$ and $pK_2 = 2{\cdot}6$ respectively (Calvin & Martell, 1953).

When chelation of a metallic ion occurs it results in the displacement of the weakly acidic protons, as for example in the reaction:

$$M^{2+} + H_2Y^{2-} \rightleftharpoons MY^{2-} + 2H^+ \qquad \ldots(1)$$

where M^{2+} represents a divalent metal cation.

Thus, when EDTA is added to sea water, chelation of the metallic ions causes a fall in pH. A typical titration curve for EDTA in sea water is shown in Fig. 1 (p. 134).

The curve of Fig. 1 was obtained by titrating sea water of 18·5‰ chlorinity containing 9×10^{-3} M disodium EDTA with 2×10^{-2} N-NaOH. The sea water contained 0·0023 equivalent per litre excess base as determined by the method of Mitchell & Rakestraw (1933). The titration was carried out in a carbon dioxide-free atmosphere. pH measurements were made by means of a glass electrode. Theoretically, two equivalents of alkali should be required to neutralize the EDTA. The discrepancy between the theoretical and the observed end-point is accounted for by the excess base present in the sea water.

The shape of the titration curve obtained is reminiscent of a strong acid–strong base titration and the effect of additions of EDTA to sea water followed by neutralization with alkali is comparable with the results of adding an equivalent quantity of any strong acid. It should be noticed that, due to the operation of the equilibrium (1), the system acts as a pH buffer in the region of pH 4–5. For most biological purposes, however, the pH of EDTA-treated sea water will be restored to pH 7 or above, and in this region the EDTA system has no buffering capacity and the bicarbonate–carbonate buffering system will therefore remain virtually unaffected. The pH of EDTA-treated sea water may therefore be adjusted by considering the EDTA to be acting as a strong acid and adjusting the resultant carbonate alkalinity and partial pressure of carbon dioxide as required.

These considerations apply only when an excess of strongly chelating cations is present. In sea water the concentration is reached at about $6{\cdot}0 \times 10^{-2}$ M EDTA. At higher EDTA concentrations the system is more complex and the buffer capacity of the sea water will be affected at physiological pH values.

THEORETICAL TREATMENT OF SYSTEM AND DERIVATION OF FORMULAE

When EDTA is added to sea water a series of equilibria of the following type operate:

$$M_1^{2+} + Y^{4-} \rightleftharpoons M_1Y^{2-}$$

$$M_2^{2+} + Y^{4-} \rightleftharpoons M_2Y^{2-},$$

etc.,

where M^{2+} represents the divalent chelating cations present. In addition, the four dissociations of EDTA must be considered. At pH values above about 3–4, the concentrations of H_4Y and H_3Y^{1-} are not appreciable and the first and second dissociations can therefore be neglected for most biological purposes.

For multi-equilibria systems of this type it is possible to write a number of simultaneous equations.

Thus: $$\frac{[M_1Y^{2-}]}{[M_1^{2+}]\,[Y^{4-}]} = K_{M_1}, \quad \frac{[M_2Y^{2-}]}{[M_2^{2+}]\,[Y^{4-}]} = K_{M_2}, \quad \text{etc.},$$

where the quantities in brackets represent concentrations, and by rewriting the analogous equations for the third and fourth dissociations of EDTA in the form

$$\frac{[HY^{3-}]}{[H^+]\,[Y^{4-}]} = K_A \quad \text{and} \quad \frac{[H_2Y^{2-}]}{[H^+]^2\,[Y^{4-}]} = K_B,$$

where $K_A = 1/K_4$ and $K_B = 1/K_3K_4$, K_3 and K_4 being the dissociation constants for the third and fourth dissociations of EDTA. In addition we have the mass equations:

$$[\text{Total } M_1] = [M_1^{2+}] + [M_1Y^{2-}]$$

$$[\text{Total } M_2] = [M_2^{2+}] + [M_2Y^{2-}]$$

etc.

and $$[\text{Total } Y] = [M_1Y^{2-}] + [M_2Y^{2-}] + \text{ etc. } + [H_2Y^{2-}] + [HY^{3-}] + [Y^{4-}].$$

In general if a system consisting of n competing ions is considered it is possible to set up $(2n+1)$ simultaneous equations. Providing the values of the stability constants (K_{M_1}, K_{M_2}, etc.) of the metal chelates, the values of the dissociation constants of EDTA (K_3 and K_4) and the total concentrations of EDTA (Total Y) and of the chelating cations (Total M_1, Total M_2, etc.) are known, then the system of equations contains $(2n+1)$ unknowns (i.e. M_1^{2+}, M_1Y^{2-}, M_2^{2+}, etc.) and may be solved for any of these.

When the case of EDTA in sea water is considered the value of n is large and the system of simultaneous equations becomes very cumbersome to solve by direct means. The following alternative treatment was therefore used.

Let $\dfrac{[M_1^{2+}]}{[Y^{4-}]}=a$, then $[M_1^{2+}]=a[Y^{4-}]$.

Similarly $[M_2^{2+}]=b[Y^{4-}]$,

etc.

Also let $\dfrac{[M_1Y^{2-}]}{[Y^{4-}]}=\alpha$, then $[M_1Y^{2-}]=\alpha[Y^{4-}]$.

Similarly $[M_2Y^{2-}]=\beta[Y^{4-}]$.

And let $\dfrac{[H_3Y^{3-}]}{[Y^{4-}]}=\lambda$, then $[HY^{3-}]=\lambda[Y^{4-}]$,

and $[H_2Y^{2-}]=\mu[Y^{4-}]$.

And let $\dfrac{1}{[Y^{4-}]}=f$.

Then from the equations for the stability constants of the metal chelates and for the dissociation constants of EDTA

$$K_{M_1}=f(\alpha/a),\quad K_{M_2}=f(\beta/b),\quad \text{etc.}$$

Also
$$K_A[H^+]=\lambda \quad \text{and} \quad K_B[H^+]^2=\mu.$$

Now
$$[\text{Total } M_1]=[M_1^{2+}]+[M_1Y^{2-}]$$
$$=a[Y^{4-}]+\alpha[Y^{4-}].$$
$$f[\text{Total } M_1]=a+\alpha$$
$$=\alpha+f\alpha/K_{M_1}$$
$$\alpha=\frac{f[\text{Total } M_1]}{1+f/K_{M_1}}$$

Similarly
$$\beta=\frac{f[\text{Total } M_2]}{1+f/K_{M_2}},$$

etc.

Now

$$[\text{Total } Y] = [M_1Y^{2-}] + [M_2Y^{2-}] + \text{ etc. } + [\text{H}_2Y^{2-}] + [\text{H}Y^{3-}] + [Y^{4-}]$$
$$= \alpha[Y^{4-}] + \beta[Y^{4-}] + \text{ etc. } + \mu[Y^{4-}] + \lambda[Y^{4-}] + [Y^{4-}].$$

$$f[\text{Total } Y]\alpha + \beta + \text{ etc. } + \mu + \lambda + 1.$$

$$f[\text{Total } Y] = \frac{f[\text{Total } M_1]}{1 + f/K_{M_1}} + \frac{f[\text{Total } M_2]}{1 + f/K_{M_2}} + \text{ etc. } + K_A[\text{H}^+] + K_B[\text{H}^+]^2 + 1.$$

This equation may be solved for f. It will be seen that the function f is by definition the reciprocal of the concentration of the fully dissociated form of EDTA (i.e. Y^{4-}). In a system consisting of several cations each competing for the Y^{4-} anion, it is the concentration of this ion, together with the total amount of a particular metal species present and the appropriate stability constant, which determines the equilibrium concentration of the unchelated metal ions. All the chelating cations present will affect to a greater or less extent the value of f for a certain concentration of EDTA. When the general equation has been solved for f for a particular case the concentration of Y^{4-} can be calculated. Substitutions of this value in the equation

$$[M^{2+}] = \frac{[\text{Total } M]}{K_M[Y^{4-}] + 1}$$

together with the appropriate values of [Total M] and K_M allows the calculation of $[M^{2+}]$.

The general equation for f is only directly applicable to a system of any number of competing metallic ions which react with Y^{4-} to give chelates of the type MY^{2-}. If chelates of other structure are found the form of the terms in the general equation corresponding to these chelates alters. For such chelates it is in general a simple matter to recast the equilibrium constant equations so that they can be incorporated in the general formula.

As an example we may consider the case of calcium. This forms chelates with both the Y^{4-} and $\text{H}Y^{3-}$ anions of EDTA according to the following reactions (Calvin & Martell, 1953):

$$\text{Ca}^{2+} + Y^{4-} \rightleftharpoons \text{Ca}Y^{2-}$$

$$\text{Ca}^{2+} + \text{H}Y^{3-} \rightleftharpoons \text{CaH}Y^{1-}.$$

Hence $$\frac{[\text{Ca}Y^{2-}]}{[\text{Ca}^{2+}][Y^{4-}]} = K_1 \quad \text{and} \quad \frac{[\text{CaH}Y^{1-}]}{[\text{Ca}^{2+}][\text{H}Y^{3-}]} = K_2.$$

The stability constants for both reactions are known and from these and the appropriate proton association constant of EDTA

$$K_A = \frac{[\text{H}Y^{3-}]}{[\text{H}^+][Y^{4-}]}$$

may be derived a fourth constant K′:

$$K' = \frac{[\mathrm{CaH}Y^{1-}]}{[\mathrm{Ca}^{2+}]\,[Y^{4-}]\,[\mathrm{H}^+]} \quad \text{where} \quad K' = K_2 \times K_A.$$

The equations for K_1 and K' are now in a suitable form for manipulation by the procedure used in deriving the general formula. It can be shown that this substitution results in two terms

$$\frac{f[\text{Total Ca}]\,K_1}{f + K_1 + K'\,[\mathrm{H}^+]} \quad \text{and} \quad \frac{f[\text{Total Ca}]\,K'[\mathrm{H}^+]}{f + K_1 + K'[\mathrm{H}^+]}$$

in place of the usual single term of the form $\dfrac{f\,[\text{Total } M]}{1 + f/K_M}$ as in the case of ions forming only one chelate.

Insertion of these terms in the general formula and solving for f enables each term to be evaluated and hence the concentration of Ca^{2+}, $\mathrm{Ca}Y^{2-}$ and $\mathrm{CaH}Y^{1-}$ calculated for a specific case. Calculation shows that in sea-water solutions of EDTA, formation of the $\mathrm{CaH}Y^{1-}$ chelate is negligible at pH values of 5 and above. At pH 4 the influence of $\mathrm{CaH}Y^{-1}$ formation on the value of f is only slight and for most purposes can be neglected. This approximation is only valid if the concentration of $\mathrm{H}Y^{3-}$ is kept low by the presence of a stoichiometric excess of strongly chelated cations. If appreciable formation of $\mathrm{CaH}Y^{-1}$ does occur it results in a reduction in the concentration of Ca^{2+}.

The general formula includes terms to allow for the effect of the third and fourth dissociation constants of EDTA. If systems at pH values below 4 are to be considered the first and second dissociations will become important. In such cases the pH-dependent terms in the general formula must be extended to include the terms $+K_C[\mathrm{H}^+]^3$ and $+K_D[\mathrm{H}^+]^4$, where

$$K_C = \frac{1}{K_2 K_3 K_4} \quad \text{and} \quad K_D = \frac{1}{K_1 K_2 K_3 K_4}.$$

APPLICATION OF THE GENERAL FORMULA TO SEA WATER CONTAINING EDTA

The constants quoted in Table 1 are the apparent stability constants determined in solutions of known ionic strength ($\mu = 0{\cdot}1$). These constants are related to the thermodynamic dissociation constants K by the relationship

$$K = K' \;\frac{\gamma M Y^{2-}}{\gamma M^{2+} \times \gamma Y^{4-}},$$

where K' is the apparent dissociation constant and γMY^{2-}, γM^{2+} and γY^{4-} are the activity coefficients of the metal–EDTA anion, the metal cation and the fully dissociated quadrivalent EDTA anion respectively.

Due to the nature of the ions relatively large variations in the activity coefficients and the apparent value of K with ionic strength can be expected. The reported constants are not therefore strictly applicable to the equilibria set up in EDTA solution in sea water. There is insufficient data for a rigorous treatment to assess the variations in the individual apparent constants with variations of ionic strength. It seems justifiable, however, to attempt an approximate treatment on the basis of the data available.

TABLE 1. STABILITY CONSTANTS OF THE EDTA CHELATES OF SOME BIOLOGICALLY IMPORTANT IONS (CALVIN & MARTELL, 1953)

Na^+	1·66	Mn^{2+}	13·4
Sr^{2+}	8·36	Co^{2+}	16·1
Mg^{2+}	8·69	Zn^{2+}	16·1
Ca^{2+}	10·59	Cu^{2+}	18·3
Ca^{2+}	3·51*		

Unless otherwise stated the values given are the log K values for the MY^{2-} chelate.

$$\star K = \frac{CaHY^{-1}}{Ca^{2+}\ HY^{-3}}.$$

Carini & Martell (1952) have investigated the changes with ionic strength of the apparent stability constant of the calcium–EDTA chelate. Their results show that in sea water of 19‰ Cl the apparent stability constant will be reduced by about a power of 10 compared with its value at $\mu = 0\cdot1$. Variations will also be caused in the values of the apparent dissociation constants of EDTA, but these can be neglected for the present approximation.

It seems reasonable to suppose that the variation in the activity coefficients γMY^{2-}, γM^{2+} with changes in ionic strength will be of the same order for all divalent metallic cations and as an approximation the apparent stability constants to be used in sea-water solutions can therefore be reduced by a similar factor. This approximation can only be justified in the case of equilibria involving divalent cations and the quadrivalent EDTA anion. As will be seen from what follows below, this limitation still allows the calculation of some data of value.

The reacting cations present in sea water fall into two groups. On the one hand there are the major constituents, Na^+, Mg^{2+}, and Ca^{2+} which are present in concentrations of the order of 10^{-2}M or above, and on the other the remaining cations present in micro- or submicromolar concentrations. Application of the general formula in the specific case of the addition of a certain concentration of EDTA to sea water indicates that for any particular case the value of f is largely controlled by a relatively small number of the chelating ions present. The dominance of an ion in this role is determined by its concentration relative to the concentration of EDTA and by the value of the stability constant of its chelate. It is thus possible to ignore the effects of many of the ions when calculating the value of f for a certain concentration of EDTA. With EDTA concentrations between 10^{-2} and 10^{-6}M the influence of the chelating reactions involving the cations of magnesium and calcium are predominant. In the case of calcium only the formation of the $\mathrm{Ca}Y^{2-}$ chelate is important, since the term corresponding to the formation of $\mathrm{CaH}Y^{3-}$ has little effect on the value of *f*.

Fig. 2 shows the variation in the value of *f* when various quantities of EDTA are added to sea water at pH 8·0. The values of *f* plotted in this curve were

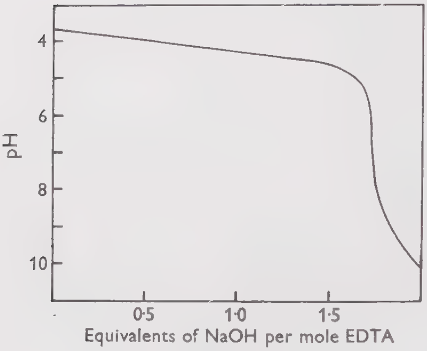

Fig. 1

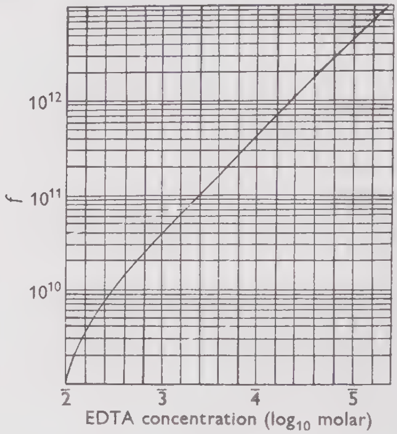

Fig. 2

Fig. 1. Titration curve for a solution of EDTA in sea water.

Fig. 2. Variation of *f* with the concentration of EDTA in sea water of 19‰ Cl at pH 8·0.

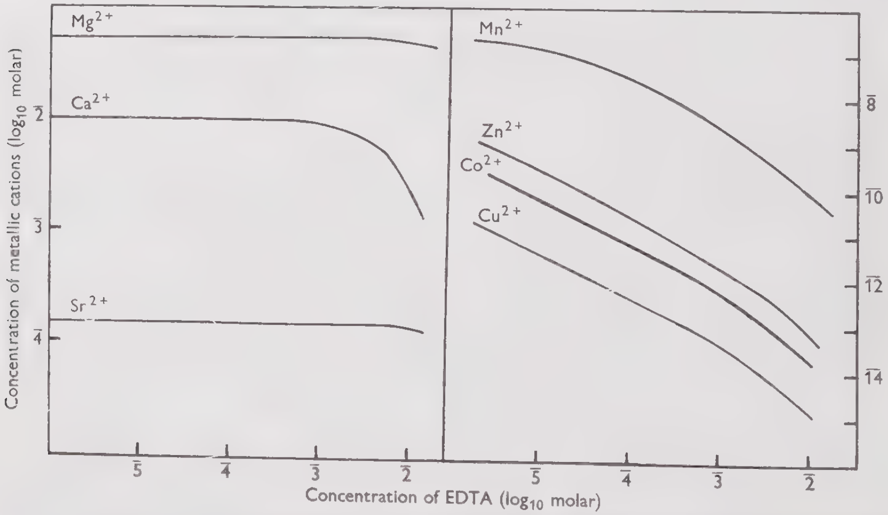

Fig. 3. Variation of the concentration of certain metallic cations in equilibrium with various concentrations of EDTA in sea water of 19‰ Cl at pH 8·0.

obtained by applying the general formula to solutions of EDTA in sea water of 19‰ chlorinity and cationic composition reported as typical by Sverdrup, Johnson & Fleming (1942). As a first approximation the influence on the value of *f* of all cations other than magnesium and calcium was neglected.

Values of *f* taken from the curve are of sufficient accuracy for many purposes. If greater accuracy is required the curve may be used for obtaining an approximate root of the equation which may then be further refined to the desired degree.

Using the value of *f* obtained from the curve in Fig. 1 the concentrations at equilibrium of the unchelated portions of several biologically important cations have been calculated for a range of EDTA concentrations. The results are shown in Fig. 3. The total concentrations of the various cations used in these calculations are taken from the same authority as the data for the construction of Fig. 2.

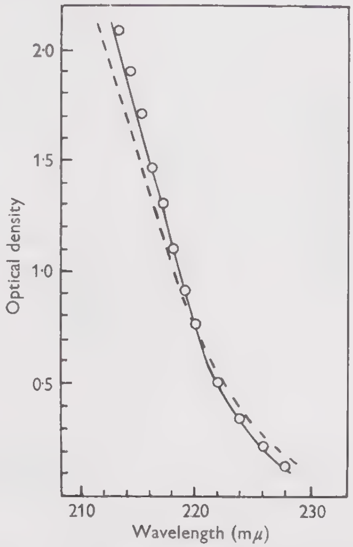

Fig. 4. Absorption spectra of 5×10^{-3} EDTA in M/15 phosphate buffer at pH 7·0 with additions of: (*a*) 5×10^{-3}M-Ca^{2+}, ———; (*b*) 5×10^{-2}M-Mg^{2+}, -----; and (*c*) 5×10^{-3}M-Ca^{2+} + 5×10^{-2} M-Mg^{2+}, ○ ○ ○. Absorption measured in 1 cm cuvette against phosphate buffer as blank using a Unicam SP 500 spectrophotometer.

An examination of the ultra-violet absorption spectrum of appropriate mixtures of calcium and magnesium ions in the presence of EDTA provides some experimental verification of the theoretical results set out in Fig. 3 above. EDTA itself absorbs strongly below about 260 mμ. Chelation with calcium or magnesium causes a shift of the absorption to shorter wavelengths. The absorption maxima cannot be determined at the concentrations which have been used and precise measurements are thus not possible. However, the curves obtained when either the calcium or magnesium chelate is used are separated (Fig. 4). If mixtures of calcium and magnesium ions in the presence of EDTA are examined, at relative concentrations such that the calcium is in stoichiometric excess of the EDTA, it is seen that the absorption spectrum of the mixture approximates to that of the calcium chelate, indicating that little magnesium is chelated under these conditions. This is as predicted by the theoretical treatment. If the concentration of EDTA is increased until it approaches the stoichiometric amount required by the calcium the absorption spectrum of the mixture starts to deviate slightly from that of the pure calcium chelate. Marked deviation, indicating appreciable formation of the magnesium chelate does not, however, occur until the EDTA concentration is equal to or exceeds the concentration equivalent to the calcium.

The case of iron has so far been omitted from the discussion and no data for the concentration of either ferrous or ferric ions which can exist in equilibrium with various concentrations of EDTA in sea water is given in Fig. 3. For several reasons the case of iron is more complicated and it will be discussed elsewhere. For most purposes, however, it is safe to assume that the presence of iron will have little effect upon the other equilibria and this is certainly true for the normal concentration range of iron found in sea water when additions of EDTA in excess of 10^{-5} M are used.

It should be noted that the calculations involved in obtaining the data for Figs. 1 and 2 have ignored the influence of all cations, other than calcium and magnesium. This approximation is reasonable within the range of EDTA concentrations considered. With concentrations of the order of 10^{-6} M the influence of the small amounts of the more strongly chelated ions such as copper becomes more important and the terms in the general formula corresponding to these ions can no longer be ignored. With concentrations of EDTA in excess of 10^{-2} M other factors—especially the pH of the system—have an increased influence in controlling the value of *f*.

THE EFFECT OF VARIATIONS OF PH ON THE EQUILIBRIA

As stated previously, chelation of cations by EDTA involves the displacement of weakly acidic protons. With certain exceptions chelation involves the completely dissociated anion of EDTA

$$M^{2+} + Y^{4-} \rightleftharpoons MY^{2-}$$

and the tendency for hydrogen ions to associate with Y^{4-}

$$\mathrm{H}^{+} + Y^{4-} \rightleftharpoons \mathrm{H}Y^{3-}$$

may thus be considered to be direct competition with metallic cation. The second proton association

$$\mathrm{H}^{+} + \mathrm{H}Y^{3-} \rightleftharpoons \mathrm{H}Y^{2-}$$

will compete indirectly. As before, the effect of the third and fourth proton association can be neglected when considering systems at pH values above 4·0.

Fig. 5 shows the variations with pH of the sum of the two pH-dependent terms in the general formula. $K_A[\mathrm{H}^+] + K_B[\mathrm{H}^+]^2$ increases rapidly with falling pH, reaching values of the order of 10^6 at pH 5. When the general formula is applied to 19‰ sea-water solutions of EDTA, the terms corresponding to the ions of magnesium and calcium, which are of dominant influence in controlling the value of *f*, are of the order of 10^7 and above. It therefore follows that at pH values of 6 and above the pH will have little effect on the equilibria set up when EDTA is added to sea water. Below a pH of 6 the two pH-dependent terms will increasingly affect the value of f and hence the equilibrium concentration of the unchelated metallic cations.

The values of the first and second proton association constants are such as to suggest at first sight that the more stable first proton association might be expected to have a marked effect, especially since the value of the association constant for this proton is of the same order as the formation constant of the calcium chelate and 10^2 greater than that of the magnesium chelate. In fact, over the pH range 8–10 when the effect of the first proton association might be expected to show, the pH has little effect on the value of f and hence on the position of the equilibria. Presumably in this pH range the mass effect of the hydrogen ions is so low compared with the 10^8 times greater amounts of calcium and magnesium that this proton association is of negligible influence in controlling the concentration of Y^{4-} compared with the chelation of calcium and magnesium.

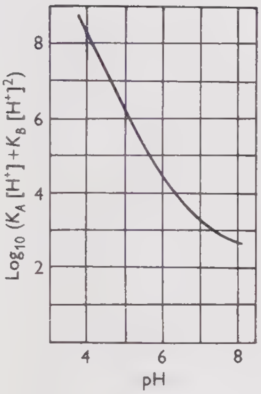

Fig. 5

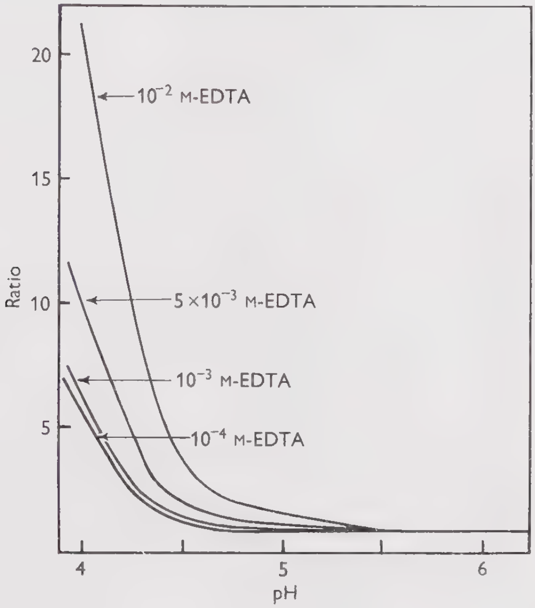

Fig. 6

Fig. 5. Variations with pH of the two pH-dependent terms in the general formula. Vertical axis shows value of $\log_{10}(K_A[H^+]+K_B[H^+]^2)$

Fig. 6. Ratio of f at various pH values to f at pH 8·0 in sea water of 19‰ Cl with various concentrations of EDTA.

As can be seen from Fig. 6 the relative effect of pH on the value of f varies with the concentration of EDTA added to the sea water. The effect is apparent at higher pH values and is of greater magnitude at the lower pH values, the more nearly the concentration of EDTA approaches the total concentration of strongly chelated cations present. With 19‰ Cl sea water this represents about 6×10^{-2} M EDTA. Additions of EDTA to 19‰ Cl sea water are in any case limited to about this concentration by solubility considerations. It is, however, possible that EDTA may be used in diluted sea water and in such cases it should be noticed that if the concentration of EDTA is equal to or exceeds the stoichiometric amount required to chelate all the strongly chelated cations, the influence of pH will be more extensive than under the conditions considered here.

The increase in the value of f caused by falling pH reflects a decrease in the concentration of uncombined Y^{4-}. This will result in an increase in the equilibrium concentration of unchelated metal cations, the increase being proportionately greater in the case of the micro-constituents than with calcium and magnesium. Thus with 10^{-2} M EDTA in 19‰ Cl sea water the concentration of Ca^{2+} at pH 4·0 will be about four times the concentration at pH 6·0 or above. In contrast, the concentration of Mn^{2+} under these conditions at pH 4·0 will be some twenty times its concentration at pH 6·0 or above. With lower concentrations of EDTA the increases in the concentration of each will be less, but relatively greater effects will still be apparent in the case of the minor constituents.

The distribution of calcium between the CaY^{2-} and the $CaHY^{1-}$ chelate will also be dependent on pH. As stated before, the formation of $CaHY^{1-}$ is negligible at pH values of 5 and above. Small amounts of $CaHY^{-1}$ are, however, formed at pH 4, but as a first approximation this has been neglected in the above discussion. When, however, appreciable formation of $CaHY^{1-}$ occurs at lower pH values it will tend to further decrease the concentration of Y^{4-}.

THE EFFECTS OF VARIATIONS IN THE SALINITY ON THE EQUILIBRIA

Variations in the salinity of the sea water to which additions of EDTA are made will affect the equilibria due to: (*a*) variations in the ionic strength of the medium, and (*b*) variations in the total concentrations of the chelating cations.

Fig. 7 shows the relative variations of f with three concentrations of EDTA in sea water of various salinities. As the salinity falls the decreasing ionic strength is associated with a rise in the apparent stability constants of the chelates and a concomitant increase in chelation. At the same time the total concentration of the chelating cations decreases, with the result that the EDTA is present in proportionately greater quantities.

As can be seen from the appropriate curves in Fig. 7 the relative importance of these two effects depends upon the EDTA concentration. With EDTA concentrations of 10^{-2}M decreasing salinity causes an immediate fall in the value of f, the influence of decreasing ionic strength being masked by the mass effect. With lower concentrations of EDTA, however, falling salinity is at first accompanied by an increase in the value of f due to the lowering of the ionic strength. When the total concentration of chelated cations falls below about 6 times the concentration of EDTA, the mass effect becomes dominant and the value of f falls sharply.

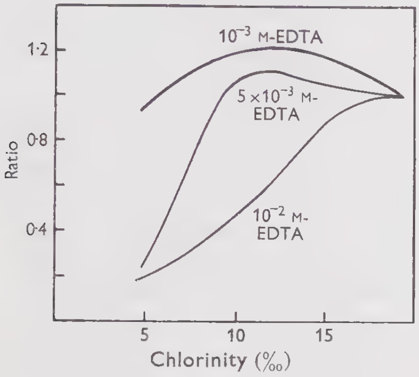

Fig. 7

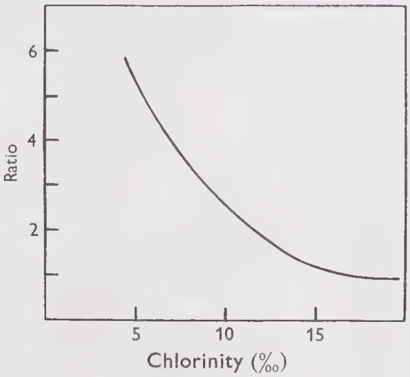

Fig. 8

Fig. 7. Ratio of f in sea waters of various chlorinities to f at 19‰ chlorinity with various concentrations of EDTA.

Fig. 8. Ratio of f at various dilutions of a 10^{-3} M solution of EDTA in sea water of 19‰ Cl at pH 8·0.

Both the increase in the value of f due to decreased ionic strength and the decrease in f caused by the mass effect are accompanied by a decrease in the concentration of unchelated metallic cations. The rise in the value of f between 19 and 10‰ Cl with 5×10^{-3} M EDTA, for example, is a reflexion of the rising stability constants which are paralleled by increasing chelation. Similarly, with the same strength of EDTA below 10‰ Cl, the rapidly falling value of f due to the mass effect is accompanied by a further decrease in metallic cation concentration, since the total concentration of the metals is decreasing.

The fall in metallic cation concentration is only slight, due to the effect of the ionic strength, but more marked due to the mass effect. The combined effect is proportionately greater with the minor constituents than with such ions as calcium and magnesium. For example, with additions of 5×10^{-3} M EDTA to sea water of 19‰ Cl the resulting concentrations of Ca^{2+} and Cu^{2+} are about $5{\cdot}5 \times 10^{-3}$ M and $4{\cdot}9 \times 10^{-15}$ M respectively. If the same addition is made to 15‰ Cl sea water the concentrations become $3{\cdot}53 \times 10^{-3}$ M and $2{\cdot}6 \times 10^{-15}$ M. With sea water of 5‰ Cl, however, the concentration of Ca^{2+} will fall to $1{\cdot}3 \times 10^{-4}$ M and that of Cu^{2+} to about $5{\cdot}8 \times 10^{-17}$ M.

It may sometimes happen that sea water containing EDTA is diluted with distilled water. In this case not only is the ionic strength of the medium reduced and the total concentration of metallic cation, but also the total concentration of EDTA is proportionately reduced. Fig. 8 shows the variation in the value of *f* when sea water of 19‰ Cl containing 10^{-3} M EDTA is diluted. The proportionate decrease in the total amount of EDTA with decreases in the concentration of the other constituents causes a progressive increase in the value of *f*. The concentration of EDTA has little effect upon the relative magnitude of the variations in *f* in dilutions of this type, and the curve may be used to correct the value of *f* when solutions of between 10^{-4} and 10^{-2} M EDTA in sea water are being considered.

THE EFFECT OF VARIATIONS IN THE TOTAL CONCENTRATION OF INDIVIDUAL CATIONS

The effect of variations in the total concentration of an individual cation will depend upon the dominance of the cation in question in controlling the value of *f*. Thus increases in the total quantity of either Mg^{2+} or Ca^{2+} will not only cause variations in the equilibrium concentrations of the unchelated portions of both ions but will also affect the equilibria of the minor constituents. In contrast, a small increase in, for example, the total amount of copper present, will have only a negligible effect on the value of *f*, and only the copper equilibrium will thus be affected. If, however, the total amount of copper is increased to a point where the appropriate term in the general formula is no longer of negligible influence on the value of *f*, then further increases in the total copper present will affect not only the Cu^{2+} equilibrium but also all the other equilibria.

The effects of increasing the total concentration of calcium in a 10^{-3} M solution of EDTA in 19‰ Cl sea water at pH 8·0 will be considered first. As the total amount of calcium present is increased the value of *f* increases. As shown in Fig. 9, *f* is directly proportional to the total concentration of calcium for a particular concentration of EDTA. The value of the proportionality constant is greater the more nearly the concentration of EDTA approaches the total concentration of strongly chelated cations. In 19‰ Cl sea water, decreases in the concentration of EDTA below 10^{-3} M have little further effect on this proportionality constant.

The increase in *f* caused by increased total amounts of calcium reflects increases in the equilibrium concentrations of all the strongly chelated cations. Table 2 gives the equilibrium concentration of several cations in a 10^{-3} M solution of EDTA in 19‰ Cl sea water at pH 8·0 appropriate to various total amounts of calcium. The concentrations of free cations increase linearly with increasing total amounts of calcium, the relative increase in each cation being approximately equal, with a given concentration of EDTA, for all cations regardless of the amount present or the value of their respective stability constants. The greater increases in *f* with higher concentrations of EDTA reflect greater increases in the equilibrium concentration of each metallic cation. Nevertheless, even with 10^{-2} M solutions of EDTA, the relative increase in free cations caused by addition of calcium remains approximately the same for a considerable range of calcium additions.

TABLE 2. EFFECT OF INCREASING TOTAL AMOUNT OF CALCIUM IN 10^{-3} M SOLUTIONS OF EDTA IN 19‰ CL SEA WATER AT pH 8·0

Total Ca present (molar)	Molar equilibrium concentrations		
	Ca^{2+}	Mn^{2+}	Cu^{2+}
$1{\cdot}0 \times 10^{-2}$	$9{\cdot}8 \times 10^{-3}$	$3{\cdot}0 \times 10^{-9}$	$3{\cdot}9 \times 10^{-14}$
$2{\cdot}0 \times 10^{-2}$	$1{\cdot}9 \times 10^{-2}$	$6{\cdot}0 \times 10^{-9}$	$7{\cdot}8 \times 10^{-14}$
$5{\cdot}0 \times 10^{-2}$	$4{\cdot}9 \times 10^{\ 2}$	$1{\cdot}5 \times 10^{-8}$	$2{\cdot}0 \times 10^{-13}$

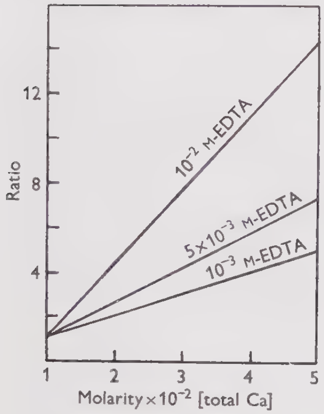

Fig. 9

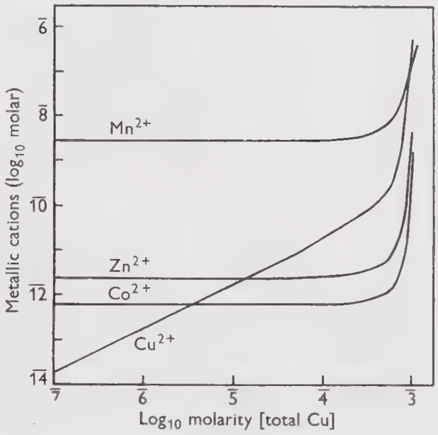

Fig. 10

Fig. 9. Ratio of *f* at various total calcium concentrations to *f* at 10^{-2} M total calcium in 19‰ Cl sea-water solutions of EDTA at pH 8·0.

Fig. 10. Variation in the concentration of certain metallic cations in 10^{-3} M solutions of EDTA in 19‰ Cl sea water at pH 8·0 with additions of various amounts of copper.

In contrast, if the total concentration of a minor constituent is increased, small increases have no appreciable effect on the value of *f* and only the concentration of the free cations of the metal in question will increase. When the total concentration of such a minor constituent is increased above a certain critical value, however, the corresponding term in the general formula will become of significance in controlling the value of *f*.

Table 3 gives the values of *f* appropriate to several total concentrations of some biologically important minor constituents. The concentration at which a particular metal will start to affect the other equilibria and the magnitude of the effect produced depend upon the stability constant of the chelate of the metal in question. Thus the other equilibria will be affected sooner by increases in the total amount of copper than by increases in less firmly bound metals such as manganese.

TABLE 3. VARIATIONS OF f IN 10^{-3}M EDTA SOLUTIONS IN 19‰ CL SEA WATER AT pH 8·0 WITH CHANGES IN THE TOTAL AMOUNTS OF CERTAIN BIOLOGICALLY IMPORTANT MINOR CONSTITUENTS

Total concentration of metal (molar)	f		
	Manganese	Zinc	Copper
10^{-5}	$3{\cdot}9 \times 10^{10}$	$3{\cdot}9 \times 10^{10}$	$3{\cdot}9 \times 10^{10}$
10^{-4}	$3{\cdot}9 \times 10^{10}$	$3{\cdot}9 \times 10^{10}$	$4{\cdot}2 \times 10^{10}$
5×10^{-4}	$7{\cdot}9 \times 10^{10}$	$8{\cdot}1 \times 10^{10}$	$8{\cdot}2 \times 10^{10}$
10^{-3}	$3{\cdot}5 \times 10^{11}$	$7{\cdot}2 \times 10^{10}$	$9{\cdot}2 \times 10^{13}$

Fig. 10 shows the effect of increases in the total copper in a typical solution of EDTA in sea water. Increases in the total amount of copper present up to concentrations of about 10^{-5}M cause proportional increases in the concentration of Cu^{2+}. At higher concentrations increasingly disproportionate increases in Cu^{2+} concentrations are caused until, at concentrations of about 5×10^{-4}M copper, further increases result in a very rapid increase in the Cu^{2+} concentration. The concentration of the other metallic cations is not appreciably affected until the total amount of copper in the system is in excess of 10^{-4}M. With total amounts of copper in excess of 5×10^{-4}M the concentration of all the other cations increases very rapidly. There will be similar effects on the concentration of Mg^{2+} and Ca^{2+}.

I am indebted to Mr Lloyd G. Chambers of the Department of Mathematics, University College of North Wales, for advice and a valuable discussion.

SUMMARY

A general formula has been derived for the quantitative treatment of systems involving a number of interdependent equilibria. The formula has been applied specifically to the equilibria set up between metallic cations and their chelates when EDTA is added to sea water. This system involves competition for the fully dissociated quadrivalent anion of EDTA (the principal ion of EDTA which forms complexes) by the metallic cations and by the hydrogen ions present. The equilibrium concentration of a metallic cation in any particular case will depend upon the equilibrium concentration of the quadrivalent anion of EDTA, in the sense that the higher the concentration of this ion the lower will be the concentration of the metallic cation. The general formula allows the calculation of the equilibrium concentration of the quadrivalent EDTA anion, and from this the concentrations at equilibrium of each metallic cation can be readily found.

When solutions of EDTA in typical sea waters are considered quantitatively, it becomes apparent that relatively few of the metallic cations present contribute significantly to determining the equilibrium concentration of the EDTA quadrivalent anion. The mass action effect of the relatively large concentrations of calcium and magnesium in sea water causes these ions to be dominant in controlling the equilibria. Consequently, changes in the total amounts of either of these molecular species cause marked changes not only in the equilibria in which they are directly involved but also affect the other equilibria. In contrast, changes within certain limits in the total concentration of other metal species have but little effect on the equilibrium concentration of the EDTA quadrivalent anion, and so affect only the equilibrium involving the metal ion in question. The limits within which this applies are controlled by the concentration at which the mass effect of the metal species in question becomes great enough to cause that particular equilibrium to have a significant effect in controlling the concentration of the EDTA quadrivalent anion. The concentration at which this occurs depends in turn upon the magnitude of the stability constant of the metal–EDTA chelate in question. The more stable the chelate, the lower the upper limit of the concentration range over which the total amount of a metal may be altered without appreciably affecting the other equilibria.

Variations in the amount of EDTA present affect all the equilibria. With lower amounts of EDTA (up to about 10^{-4} M) there is an approximately inverse proportionality between the total amount of EDTA present and the concentration of unchelated metallic cations. As the total amount of EDTA approaches a value stoichiometrically equivalent to the total amount of divalent cations present (i.e. about 6×10^{-2} M in a typical sea water), there is a progressive disproportionate decrease in the equilibrium concentration of each metallic cation.

An examination of the effects of pH on the system shows that in sea-water solutions of EDTA, pH will have little effect on the equilibrium concentrations of free metal ions within the range pH 6–10. Below pH 6 increases in the metallic cation concentration will occur. These increases will be proportionately greater in the case of the minor constituents of sea water. The relative pH-independence of the system above pH 6 is limited to systems in which divalent metal cations are in stoichiometric excess of the EDTA.

The equilibria in EDTA–sea water systems are dependent upon the ionic strength of the medium and hence on the salinity. A fall in salinity with its concomitant decrease in ionic strength is associated with increased chelation.

Graphs are presented showing the variations of the reciprocal of the concentration of the quadrivalent EDTA anion (f) with variations in those factors affecting the equilibria which are summarized above. These will allow an approximate evaluation of the ionic concentrations of metallic cations in many cases which may be encountered in biological work. For more precise calculations the interpolated values of f may be used as a first approximation to the root of the appropriate equation for f, which may then be refined to whatever degree is desired.

The general procedures used are applicable to many biological fluids other than sea water, and for similar systems of competing equilibria other than the formation of complexes.

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NEMATODES PARASITIC ON SEA WEEDS OF THE GENERA *ASCOPHYLLUM* AND *FUCUS*

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(Text-figs. 1–3)

In 1892 Barton described galls found on the sea weed *Ascophyllum nodosum* from the Isle of Cumbrae, west coast of Scotland, and from Stonehaven, east coast of Scotland. The affected areas on the thallus of the plant appeared as swellings covered with small rounded nodules, and were almost invariably confined to the parts of the thallus just above or below the air vesicles. A transverse section through the swellings showed each nodule as a more or less hollow space containing numerous nematodes, specimens of which were sent to J. G. de Man, who (1892) described the worm as a new species, *Tylenchus fucicola*, the first-known marine tylenchid.

On the basis of de Man's description Cobb (N. A. Cobb in M. V. Cobb, 1933) erected the genus *Halenchus* for this species. Since then, other species of *Halenchus* have been described, but only as free-living forms.

From time to time galls on other sea weeds have been reported, but attributed to other animal agents, bacteria and fungi.

During visits by the author to the Laboratory of the Marine Biological Association at various times during 1955–57 galls were found very commonly on *Ascophyllum nodosum* in the Plymouth area (Wembury Bay). They agree well with Barton's description and contain numerous specimens of a nematode, which appears to be identical with de Man's form. Similar galls were also found at Croyde Bay on the north Devon coast in August 1955 (Fig. 1 A).

Galls were also found on the sea weed *Fucus vesiculosus* (Fig. 1 B) from Wembury Bay, Devon, and at Hannafore, Looe, Cornwall. They were found only on the older growths of the plant and occurred mainly on the stipes, and less frequently on the base of the fronds. The galls are quite noticeable and are usually a little lighter in colour than the rest of the stipes, but are not so large as some of those seen on *Ascophyllum*. The surface of the swellings is rugose and not so papillate as the galls on the other plant. When the galls were opened nematodes were found, but there were only a few in each in contrast with the galls on *Ascophyllum* which contain large numbers. At first the worms were thought to be identical with *Halenchus fucicola*, except that the anterior end appeared rather larger. On closer examination, however, several small differences have been noted and are considered to be of sufficient significance to warrant the recognition of a distinct species.

Frequently non-tylenchid nematodes are found in the galls as well as the marine mite *Rhombognathus* (*Rhombognathopsis*) *seahami* (Hodge), kindly identified by Dr G. O. Evans. The nematodes appear to represent a variety of species no one of which appears to be consistently present; thus leading one to conclude that they are not the causal agents.

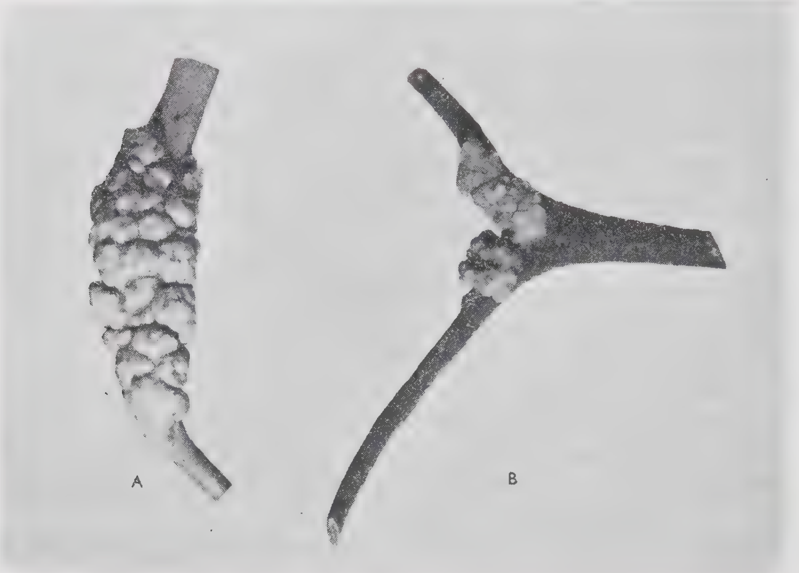

Fig. 1. Galls on (A) *Ascophyllum nodosum*, and (B) *Fucus vesiculosus*. (Approx. natural size.)

During a one-day visit to Lyme Regis, Dorset, in July 1957, galls were seen on the stipes of *Fucus vesiculosus*, and in some plants there were also galls on the fronds. In this locality galls were also seen on the stipes of *F. serratus*. On examination, these were found to be very similar to those on *F. vesiculosus*, and contained the same species of nematode.

The examination of the nematodes in the above material has been based on living specimens, as well as on others killed by heat, fixed in formalin and subsequently cleared in lactophenol and glycerine.

A redescription is given below of *Halenchus fucicola*, together with a description of the new species, *H. dumnonicus*.[1]

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[1] From the Dumnonii, the British tribe that inhabited the south-west.

Halenchus fucicola (de Man, 1892) Cobb, 1933

Tylenchus fucicola de Man, 1892. *Festschr. zum Siebenzigsten Geburtstag Rudolf Leuckart*, pp. 121–5.
Anguillulina fucicola, Goodey, 1932. *J. Helminth.* Vol. 10, p. 27.
Halenchus fucicola, Cobb, 1933. *J. Parasit.* Vol. 20, p. 94.

Type host. In galls on the sea weed *Ascophyllum nodosum* Le Jol.

Material studied. From galls on *A. nodosum*, Wembury Bay, Devon, August 1955, June 1956, and May 1957.

Dimensions. Given in Table 1.

Long slender worms, gradually tapering at the extremities, with a characteristic hook-like tail, bent ventrally in both sexes. The cuticle is faintly transversely striated. The striations, which appear to be absent on the head-region, being about 1 μ apart. Lateral fields are present, which are about one-eighth of the body thickness; they appear to start at about half-way between the anterior end of the body and the excretory pore and can be traced to the tail region.

The head is marked off by a slight constriction, this latter being about 6 μ in diameter. There appear to be no distinct lips, the head-framework being divided into six sectors defined by faint ridges. Papillae are present on the two subdorsal and two subventral sectors, and the openings of the amphids are on the lateral ones. The amphidial pouches are situated laterally on either side of the stylet (Fig. 2C). The stylet is fairly well developed (15–16 μ long in the males and 15–18 μ long in the females) and is divided into two portions, a cylindrical posterior portion, with three well-pronounced basal knobs, and a conical anterior portion (Fig. 2C, D).

The excretory pore and its duct are well defined and characteristic, being heavily cuticularized. The pore is situated ventrally about one-eighth of the body length from the anterior end and the duct runs spirally in a posterior direction. The oesophagus is poorly developed. About mid-way along its length there is a slight swelling of the oesophageal wall associated with which is a somewhat ellipsoid widening of the lumen, probably representing a middle bulb. The lumen of the oesophagus can be seen in living specimens to have a wavy outline. The junction of the oesophagus and intestine is not clear, and was only seen in living specimens, where it occurred at about 20–25 μ anterior to the excretory pore. The granulated material of the intestine was seen to commence at this region and sometimes could be seen to run anteriorly into the oesophagus. Close behind the nerve ring, which is situated at the posterior end of the oesophagus, a dorsal oesophageal gland emerges from the wall of the oesophagus as a glandular diverticulum running alongside the intestine. This gland has a fairly well-defined nucleus and opens into the lumen of the oesophagus about 2–3 μ behind the base of the stylet. The distance of the extremity of the gland from the anterior

TABLE 1. DIMENSIONS OF *HALENCHUS FUCICOLA* (DE MAN, 1892) FROM WEMBURY BAY

Sample		Body length (μ)	Body length / Body thickness	Body length / Excretory pore	Body length / Length of tail (anus to tip)	Body length / Length of stylet	Body length / Length of spicules	V (%)
August 1955, ♂♂	Range	800–970	34·58–48·50	7·17–8·18	10·90–12·93	51·90–64·67	36·36–43·00	—
	Mean	891	38·10	7·78	11·73	58·67	40·70	—
	Standard deviation	±55·26	±7·08	±0·28	±0·63	±4·16	±2·18	—
	No. of specimens	10	10	10	10	10	10	—
August 1955, ♀♀	Range	970–1120	32·50–46·67	7·30–9·55	10·18–13·75	60·62–70·00	—	60·00–68·04
	Mean	1052·70	40·52	8·51	12·95	65·45	—	62·17
	Standard deviation	±54·50	±5·45	±0·56	±1·49	±3·31	—	±2·44
	No. of specimens	11	11	11	8	11	—	9
June 1956, ♂♂	Range	820–970	35·38–51·11	7·39–8·94	10·90–13·14	45·55–64·67	37·27–45·00	—
	Mean	883	42·06	8·11	11·63	56·50	41·68	—
	Standard deviation	±48·31	±4·69	±0·44	±0·75	±5·45	±2·12	—
	No. of specimens	10	10	10	9	10	10	—
June 1956, ♀♀	Range	980–1100	33·44–49·52	8·00–9·48	12·22–15·00	61·25–73·33	—	59·09–64·00
	Mean	1056	41·69	8·85	13·48	66·46	—	61·37
	Standard deviation	±41·35	±4·96	±0·48	±1·06	±3·55	—	±1·55
	No. of specimens	10	10	10	6	10	—	10
May 1957, ♂♂	Range	920–1060	35·92–45·91	7·36–7·92	11·05–12·80	57·50–67·33	40·00–55·71	—
	Mean	963	42·20	7·67	11·87	62·18	44·08	—
	Standard deviation	±43·72	±3·57	±0·17	±0·57	±3·19	±5·07	—
	No. of specimens	10	10	9	8	10	9	—
May 1957, ♀♀	Range	1040–1430	33·94–49·26	7·47–8·22	12·18–14·05	61·17–79·44	—	59·61–65·76
	Mean	1159	42·51	7·84	12·99	68·43	—	63·76
	Standard deviation	±46·80	±4·98	±0·28	±0·71	±6·17	—	±1·99
	No. of specimens	10	9	8	8	10	—	8

The ratio body length/excretory pore has been used instead of the usual '*b*' (body length/oesophagus) because of the difficulty of determining the posterior limit of the oesophagus. *V* is the distance of the vulva from the anterior end of the body expressed as a percentage of the body length.

TABLE 2. DIMENSIONS OF *HALENCHUS DUMNONICUS* SP.NOV. FROM WEMBURY BAY, 1955, 1956, 1957, AND HANNAFORE, LOOE, 1956

Sample		Body length (μ)	Body length / Body thickness	Body length / Excretory pore	Body length / Length of tail (anus to tip)	Body length / Length of stylet	Body length / Length of spicules	V (%)
♂♂	Range	850–1200	33·85–46·15	6·44–7·84	9·56–13·33	39·54–57·14	35·42–50·00	—
	Mean	989·10	40·09	7·20	11·12	47·57	40·45	—
	Standard deviation	±38·15	±3·27	±0·52	±1·23	±6·12	±4·26	—
	No. of specimens	11	11	10	10	11	11	—
♀♀	Range	900–1480	30·86–47·62	6·43–9·31	9·69–11·95	42·86–61·36	—	64·76–88·89
	Mean	1091·20	39·41	7·52	10·96	50·76	—	69·94
	Standard deviation	±166·55	±4·79	±0·76	±0·75	±5·08	—	±6·20
	No. of specimens	16	16	12	10	16	—	12

end of the body varies from 155 to 253 μ. The intestine terminates in a short rectum.

Deirids were not seen, while phasmids are present on the tail in the posterior region of the lateral caudal alae in the males, and occur in the region about half-way between the anus and the tip of the tail in the females.

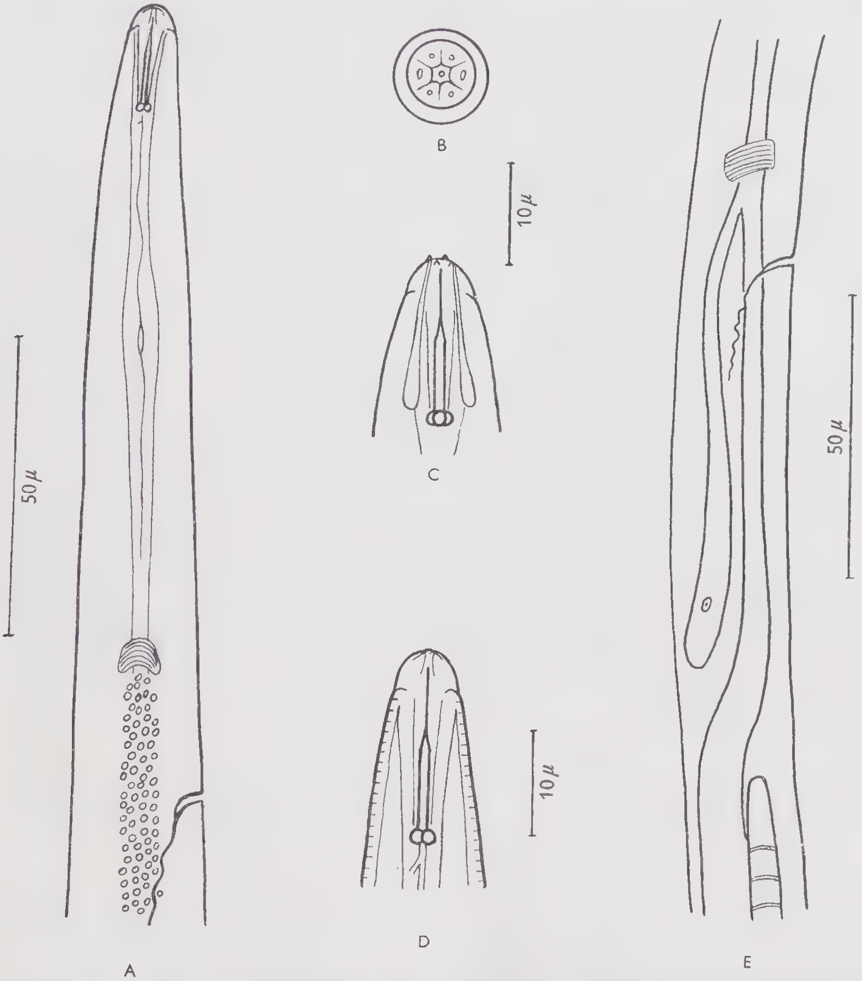

Fig. 2. A, *Halenchus fucicola*, anterior end; B, *H. fucicola*, head (*en face*); C, *H. fucicola*, head (ventral view showing papillae and amphids) (B and C to same scale); D, *H. fucicola*, head (lateral view); E, *H. dumnonicus*, posterior oesophageal region (lateral view).

Male. Testis single and straight, commencing at the anterior region of the intestine, opening without any apparent constriction into a vas deferens, which ends in a short ejaculatory duct. Spicules paired and equal, arched, with slight variations, 20–24 μ in length (Fig. 3C, F and G). Gubernaculum trough-like, about 6–7 μ in length in a lateral view. Lateral alae present, commencing just anterior to proximal end of spicules and extending to about mid-way between cloaca and tip of tail.

Female. Ovary single and straight, anterior to vulva, and commencing at anterior region of intestine. Oviduct well differentiated and leading to a uterus, the cell walls of which are polygonal. Vulva with slightly elevated lips situated posterior to middle body region. The short vagina is directed dorsally. The eggs measure 75–80 μ by 23–24 μ; only one has been seen at a time in the uterus.

Larvae: Found at various stages of development in the galls of *Ascophyllum*, and they too have the characteristic hook-like tail. One of the smallest specimens measured 330 μ in length, 12 μ in maximum thickness with a stylet 11 μ long. Others measured from 420 to 730 μ in length; 14 μ in maximum thickness; stylet 12–13 μ long; tail (anus to tip of tail) about 60 μ long.

Geographical distribution. East and west coast of Scotland: Stonehaven and Isle of Cumbrae (Barton, 1892); Seamill, West Kilbride, Ayrshire (de Man, 1892). Port Erin, Isle of Man (Goodey, 1932). North and south coast of Devon (present author). Heligoland (Schuurmans Stekhoven, 1935; Bresslau & Schuurmans Stekhoven, 1940). Coast of Norway (Allgén, 1934*b*). Woods Hole, Mass., U.S.A. (Chitwood, 1951).

Halenchus fucicola has also been reported as parasitic on *Ceramium rubrum* from Heligoland by Schuurmans Stekhoven (1935, p. 155). This statement appears to be based on the one immature male specimen found on *C. rubrum* from Heligoland by Bresslau & Schuurmans Stekhoven (1940, p. 69, pl. xiv, fig. 79*a–b*) where a description and figure of the specimen is given. No mention of galls present on the plant is given and presumably the specimen was found free-living among the algae.

Halenchus dumnonicus sp.nov.

Type host. In galls on the sea weed *Fucus vesiculosus* L.

Type locality. Wembury Bay, south Devon.

Other host. F. serratus L.

Material studied. From galls on *F. vesiculosus*, Wembury Bay, south Devon, August 1955, June 1956, May 1957; and Looe, Cornwall, June 1956.

Holotype. ♂, B.M. No. 1955, 11.1.301. *Allotype.* ♀, B.M. No. 1955, 11.1.302. *Paratypes:* B.M. No. 1955, 11.1.303–311 (5 ♂♂, 4 ♀♀); 1957, 8.1.1–12 (6 ♂♂, 6 ♀♀). Host material registered under B.M. No. 1955, 11.1.312–325. 1957, 8.1.13–16.

Dimensions. Given in Table 2.

The shape of the body is very similar to that of *Halenchus fucicola* except that the anterior end is much more strongly developed in proportion to the total length of the worm, and the stylet, although of similar shape to the other form, is much longer (20–22 μ long in the males and 20–25 μ long in the females). The tip of the tail is again hook-like, bent ventrally in both sexes.

The transverse striations of the cuticle, lateral fields, structure of the head and papillae are the same as in *H. fucicola*.

The excretory pore and its duct bear a considerable resemblance to those seen in *H. fucicola*, although the duct does not appear to be quite so heavily cuticularized and is situated ventrally about one-eighth of the body length from the anterior end. The structure of the oesophagus is also similar to that of the other species and the nerve ring is situated at the posterior end of it. The connexion of the oesophagus with the intestine is not clear in this species also and occurs just posterior to the nerve ring, about 20–25 μ anterior to the excretory pore. The dorsal oesophageal gland usually appears more slender than it does in the previous species, but this structure is somewhat variable in size. In some of the preserved and cleared specimens the connexion of the gland and the wall of the oesophagus is more easily seen (Fig. 2E). The opening of the gland into the lumen of the oesophagus occurs at about 2–3 μ from the base of the stylet. The distance of the extremity of the gland from the anterior end of the body is about 200 μ. The intestine is as in *H. fucicola*.

Deirids have not been seen. The phasmids have not been seen in this form.

Male. Testis and vas deferens as in *H. fucicola*. Spicules are longer, stouter and differ in shape from those in the other form (21–27 μ in length). In lateral view a well-pronounced protuberance can be seen raised in the form of a hump on the ventral side in mid-region; towards the proximal end there is also a greater curvature (Fig. 3D, H). Gubernaculum is trough-like, about 7 μ in length in a lateral view. Lateral alae are present as in *H. fucicola*.

Female. Ovary, oviduct and uterus similar to that in *H. fucicola*. Two specimens were found each bearing an egg; in one case the egg measured 66 by 22 μ and in the other 108 by 27 μ. Vulva with slightly elevated lips, situated posterior to the middle body region. Seen in a lateral position the vagina appears to have a greater cuticular development than in *H. fucicola* (Fig. 3E). A short post-vulvar uterine sac is present.

Larvae. Not seen.

Geographical distribution. South Devon and south Cornwall; Lyme Regis, Dorset.

The new species is a slightly larger form and differs from *H. fucicola* principally in the greater development of the stylet and in the shape of the spicules. In *H. fucicola* the males average about 900 μ in length and the females, including those bearing an egg, average just over 1000 μ. Only one specimen was found as long as 1430 μ and specimens over 1100 μ are not common. The maximum body thickness varies from 18 to 28 μ in the males and 20–33 μ in the females. In *H. dumnonicus* the males average about 1000 μ in length and the females about 1100 μ. Only two gravid specimens were

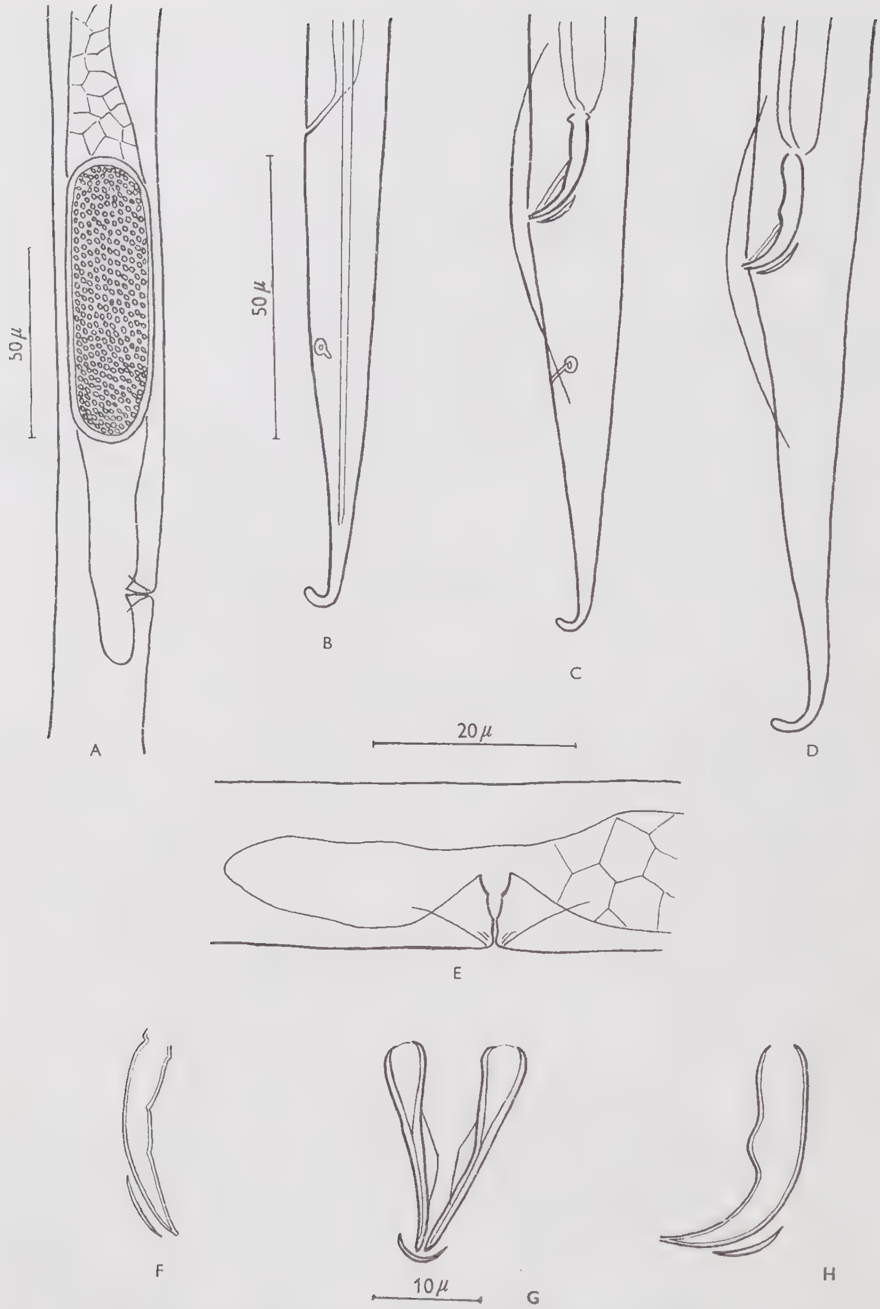

Fig. 3. A, *Halenchus fucicola*, posterior uterine region with egg; B, *H. fucicola*, female tail (showing lateral field and phasmid); C, *H. fucicola*, lateral view of male tail; D, *Halenchus dumnonicus*, lateral view of male tail (B, C and D to same scale); E, *H. dumnonicus*, posterior uterine region; F, *H. fucicola*, lateral view of spicule and gubernaculum; G, *H. fucicola*, ventral view of spicules and gubernaculum; H, *H. dumnonicus*, lateral view of spicule and gubernaculum (F, G and H to same scale).

found, each bearing one egg and measuring 1240 and 1270 μ in length respectively. The maximum body thickness varies from 20 to 31 μ in the males and 21–38 μ in the females.

The samples considered here are not random because the intention during sampling was to get some idea of the full range of size, so that the number of large and small specimens measured is probably biased in favour of such individuals. As a result of this it is valueless to compare the means of the absolute measurements. The difficulty, however, can be overcome by comparing ratios: while appreciating that ratios may also vary due to differential growth. For example, in *H. fucicola* the ratio body length/stylet length is 30 at a larval body length of 330 μ, 35 at 420 μ and 56 at 730 μ. In a sample of ten adults, mean body length 970 μ, the ratio is 59·60; and in another sample of 10 adults, mean body length 1159 μ, it is 68·43. These values were obtained by dividing a sample of twenty individuals into two halves on the basis of body length. From these figures it can be seen that the body grows at a greater rate than the stylet.

Ratios have been calculated for body length/body thickness, body length/distance of excretory pore from anterior end, body length/tail length, body length/stylet length, body length/length of spicules, and the distance of the vulva from the anterior end expressed as a percentage of the body length, i.e. vulva × 100/body length. The mean body lengths, given in the tables, are in all cases not significantly different, so that the ratios calculated against them should be strictly comparable. The ratios were calculated for all specimens and means and standard deviations calculated for them. Of these only the ratios of body length/length of stylet are significantly different in the two forms.

Due to the low number of specimens, the measurements of *H. dumnonicus* have been lumped, and it is this total which has been compared to each sample of *H. fucicola*.

As mentioned earlier, the specimens in the galls of *Fucus vesiculosus* are much scarcer, and when living specimens are removed from the galls they are usually more active than those of *Halenchus fucicola* from *Ascophyllum*. Specimens are also scarce in the galls on *Fucus serratus*.

Of the remaining species of *Halenchus*,[1] *H. dumnonicus* differs from *H. mediterraneus* (Micoletzky, 1922) in the length of the stylet, the position of the caudal alae, which do not extend so far posteriorly, and in the size and shape of the gubernaculum, which is smaller and has a wavy outline.

Finally, the new species differs from *H. mexicanus* Chitwood, 1951, in the shape of the tail, which is not hook-like.

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[1] *Tylenchus* (*Chitinotylenchus*) *zostericola* Allgén (1934*a*) found associated with *Zostera* in Holland was transferred to *Halenchus* by Chitwood (1951), but is now considered to belong to the genus *Radopholus* (see Allen, 1955).

These tylenchid nematodes occur on the fucoid algae which predominate in the middle zone of the shore, and at Wembury Bay much time has been spent by the writer looking for galls on *Fucus spiralis* and *F. serratus* without success, although on a later visit to Lyme Regis galls were seen on the latter species.

Ascophyllum nodosum and *Fucus vesiculosus* occur at approximately the same height on the shore, and at Wembury Bay galls were sometimes seen on both plant species where they were growing near to each other. It would therefore seem possible for both species of nematodes to infest either plant, since at some stage in the life cycle some of the worms presumably emerge from the galls to infest new host plants. An obstacle to the elucidation of the life history of the worms by experimental means, however, arises from the difficulty of maintaining the large brown sea weeds under laboratory conditions.

Sometimes *Halenchus fucicola* and *Halenchus* sp. are quoted as parasites of red sea weeds, *Furcellaria fastigiata*, *Chondrus crispus* and *Rhodymenia palmata*. It appears, however, that these statements are based on the reports in two other papers by Barton (1891, 1901). Barton's material of *Furcellaria* and *Chondrus*, which was found washed up at Lyme Regis in 1900, is in the algal collections of the British Museum (Natural History) and the writer has had the opportunity of examining it. Although the nematodes in the galls of these plants are in very poor condition they certainly do not appear to be tylenchids. According to Barton (1891), the nematodes from *Rhodymenia palmata* were found in galls attributed to a copepod, and in a recent paper by Harding (1954), in which a description is given of the life history of the copepod from galls on *Rhodymenia palmata*, nematodes were again reported in some of the galls.

Thanks are due to Dr H. W. Parker who suggested this line of research, to Mr W. G. Inglis and Mr S. Prudhoe for much help and advice during the course of the work, and to the staff of the Laboratory at Plymouth, especially to Dr M. W. Parke.

SUMMARY

The nematode *Halenchus fucicola* (de Man), causing galls on the thallus of the sea weed *Ascophyllum nodosum*, has been found in the Plymouth area (Wembury Bay) and at Croyde Bay on the north Devon coast, and a redescription of the worm is given. A new species of *Halenchus* (*H. dumnonicus*) causing similar growths on the sea weeds *Fucus vesiculosus* and *F. serratus* is described. This latter form is also found at Wembury Bay; at Looe, Cornwall, and at Lyme Regis, Dorset.

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THE DISTRIBUTION OF INTERTIDAL ORGANISMS ALONG THE COASTS OF THE ENGLISH CHANNEL

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(Text-figures 1–7)

INTRODUCTION

Our investigations on the distribution of barnacles (Crisp & Chipperfield, 1948; Crisp, 1950; Southward, 1950, 1951; Norris & Crisp, 1953; Southward & Crisp, 1952, 1954*a*; Bishop & Crisp, 1957, 1958) were prompted by the lack of quantitative information on the distribution of most species. During these investigations we noted that the distribution of barnacles and of other species of shore animals had certain features in common. We have therefore extended our studies to include many of the commoner intertidal organisms in the belief that we may be able to discover which are the most important of the many possible factors controlling the distribution of littoral species generally, and of barnacles in particular.

We have already described the main features of the distribution of the commoner intertidal animals along the Irish coast (Southward & Crisp, 1954*b*). The present paper, the second of a series, deals with the English and French sides of the Channel. These two coasts are most conveniently treated together for several reasons. In the first place the Channel presents a simpler picture, both hydrographically and faunistically, than that afforded by the seas bounding the other coasts of the British Isles. It therefore offers a useful introduction to further contributions which we are preparing on the British Isles.

The fauna and flora of the Atlantic coast of France will be described elsewhere by one of us (D. J. C.) in collaboration with Prof. E. Fischer-Piette.

In describing distributions along the Channel coasts we have included the whole region lying between Cape Cornwall and North Foreland on the English side and the region between Calais and Le Conquet on the French side, together with Ile d'Ouessant, the Channel Islands and the Isles of Scilly. In this area we studied some of the commoner animals and plants found on predominantly rocky shores (including large boulders, piers and breakwaters). As far as possible the dominant organisms were treated quantitatively. In previous papers (Southward & Crisp, 1954*a*, *b*, 1956) we have stressed the importance of a quantitative approach to biogeography. Some measurement of the abundance of the organisms is essential if the results are intended to be used later to check changes in distribution, and is equally important for the purpose of revealing the main controlling factors.

In two previous studies of the area, that of Moore & Kitching (1939) on barnacles and the more general work of Fischer-Piette (1936), some quantitative information is given for certain of the species we have investigated. However, Moore & Kitching investigated only the distribution of *Chthamalus stellatus*, and to a lesser extent that of *B. balanoides*, on the English side. Fischer-Piette, although he included species (especially of plants) that we ourselves did not investigate, omitted a detailed account of the common periwinkles and did not consider the top-shells. He concerned himself mainly with details of the distribution in the western half of the Channel, and considered particularly the effects of comparatively local factors such as tidal conditions and wave action. We have investigated the fauna throughout the Channel, and have tried to relate the patterns of distribution both to general trends in the physical environment and to such topographical details as seem to be significant.

The species investigated are listed in Table 1, together with brief references to their distribution outside the Channel and their periods of breeding. The species in brackets are those that were not investigated as fully as the other species, or those for which we had to rely upon previous observations. The terms of abundance employed in the table have exact quantitative meanings for each species, as explained in Table 2.

THE PHYSICAL ENVIRONMENT

The features of the environment most likely to influence the geographical distribution of the plants and animals of the shore can be divided into two groups. The primary factors include the temperature of the sea and the air, the salinity of the sea and its content of nutrient salts, and the currents and water movements of the sea. The variation of these factors in the Channel has been discussed by Russell (1953); they are readily defined and measured, though the information regarding them may not be complete or satisfactory as far as the intertidal zone is concerned. The other factors, including the degree of exposure to wave action and 'biological factors' attributed to water masses of different past history, are at present difficult even to define, let alone to measure.

Temperature

The monthly means of sea and air temperatures for February and August are shown in Fig. 1. These months represent the overall extremes of average temperature for the year. At some stations slightly greater extremes of air temperature may be found in January and July.

We have already commented (Southward & Crisp, 1954*b*) on the difficulty presented by the differences between the temperature of the sea and the air.

TABLE 1. THE SPECIES INVESTIGATED, THEIR GENERAL DISTRIBUTION AND MAIN BREEDING SEASONS*

	Abundance in Channel†		North European limits	South European (or other) limits	Months or season of main breeding period in Channel
	West	East			
Southern forms:					
Anemonia sulcata (Pennant)	A	R	N.W. Scotland	Med., Canaries	
Chthamalus stellatus (Poli)	A	N	N.E. Scotland	(W. Africa)	May–Sept.
Balanus perforatus (Bruguière)	A	N	S. Wales	(W. Africa)	July–Sept.
Patella depressa (Pennant)	A	N	N. Wales	(N. Africa)	July–Aug.
P. aspera (Lamarck)	A	N	Scotland, N.E. England	Spain	Sept.–Nov.
(*Haliotis tuberculata* (L.))	+	N	N. France	Mediterranean	July–Sept.
(*Gibbula magus* (L.))	C	O–R	N. Scotland	Mediterranean	?
G. pennanti Philippi	A	N	N. France	?	?
G. umbilicalis (da Costa)	A	C–R	N. Scotland	Mediterranean	Autumn
Monodonta lineata (da Costa)	A	N	N.W. Ireland	(Madeira)	Autumn
Littorina neritoides (L.)	A	R	Scotland, N.E. England	(N. Africa)	Winter
(*Paracentrotus lividus* (Lamarck))	+	N	N.W. Ireland, S.W. Scotland	(Azores)	Probably summer
(*Laminaria ochroleuca* (la Pylaie))	+	N	S. England	(N. Africa)	Aug.–Nov.
(*Saccorhiza polysichides* (Lightf.))	+	N	W. Norway, N. Scotland	(N. Africa)	Probably all year
(*Bifurcaria rotunda* (Huds.))	+	N	Ireland, S. England	(N. Africa)	Spring
Intermediate forms:					
Actinia equina L.	A	A	Murman Sea	(Africa)	Viviparous; probably all year round
Verruca stroemia (O. F. Müller)	A	C–R	S. Norway, Iceland	Mediterranean	Nov.–May
Balanus improvisus Darwin	A	A	Baltic	(Tropics)	May–Sept.
Elminius modestus Darwin	A	A	S.W. Scotland	France	May–Oct.–Jan.
Hemioniscus balani Spence-Bate	A	R	60–62° N. in *B. balanus*	?	?
Patella vulgata L.	A	A	N. Norway	Mediterranean	Oct.–Mar.
Gibbula cineraria (L.)	A	A	N. Norway	Mediterranean	Probably winter
(*Calliostoma zizyphinum* (L.))	+	+	S. Norway	Mediterranean	Probably winter and spring
Littorina obtusata (L.)	A	A	Murman Sea	Portugal	Winter and spring
L. littorea (L.)	A	A	Murman Sea	Portugal	Probably all year
Fucus spiralis L.	+	+	N. Norway, Iceland	(N. Africa)	Summer
(*Himanthalia elongata* (L.))	+	N	N. Norway (not Iceland)	Spain	May–Oct.
Northern forms:					
Balanus balanoides (L.)	C–R	A	Arctic	N. Spain	Feb.–Apr.
B. balanus (L.)	N	?	Arctic	Ireland, S. Wales, S.E. England	Feb.–Apr.
B. crenatus Bruguière	A	A	Arctic	W. France	Feb.–May
Littorina saxatilis (Olivi)	A	A	Arctic	Portugal	All year
(*Laminaria digitata* (Huds.))	+	+	Arctic	N.W. Spain	All year
(*L. hyperborea* (Gunn.))	+	+	N. Norway	N.W. Spain, Portugal	Nov.–Apr.
(*L. saccharina* (L.))	+	+	Arctic	W. France	Winter, but some all year
Alaria esculenta (L.)	+	N	Arctic	W. France	Jan.–Apr.
Ascophyllum nodosum (L.)	+	+	Arctic	Spain	Spring
Fucus serratus L.	+	+	Murman Sea, White Sea	N. Spain	Autumn
F. vesiculosus L.	+	+	Murman Sea, White Sea	(N. Africa)	All year
Pelvetia canaliculata (L.)	+	+	White Sea	Portugal	June–Sept.

* Details of distribution outside the Channel and information on breeding periods have been obtained from the following works, supplemented by personal observations where available: Forbes & Hanley, 1853; Darwin, 1854; Sars, 1878; Woodward, 1880; Børgeson & Jónsson, 1908; Stephenson, 1924, 1935; Mortensen, 1927; Crofts, 1929; Nobre, 1932; Hamel, 1931–39; Fischer-Piette, 1935, 1936, 1955; Fischer-Piette & Prenant, 1956; Stephensen, 1938; Bassindale, 1941; Lami, 1941, 1943, 1954; Thorson, 1941, 1946; Station Biologique de Roscoff, 1951 *a*, *b*, 1954; Lilly *et al.* 1953; Orton *et al.* 1956; Marine Biological Association, 1957.

† For meanings of symbols refer to Table 2. Parentheses round certain species indicate that our observations were incomplete.

TABLE 2. MEANINGS OF THE NOTATION USED TO DESCRIBE THE ABUNDANCE OF CERTAIN INTERTIDAL ANIMALS

For all organisms the letters involved represent the same word: A, abundant; C, common; F, frequent; O, occasional; R, rare; and N, not found. The exact quantitative basis of these categories varies with each species, as follows:

Anemones

A	Many in almost every pool and damp place
C	Groups in pools and damp places
F	Isolated specimens in few pools
R	A small number, usually under 10, found after 30 min searching

Barnacles

For *Chthamalus stellatus*, *Balanus balanoides* and *Elminius modestus*, we have usually recorded numbers per square centimetre of rock. For these and other species except *Balanus perforatus* the categories have the following meanings:

A	More than 1 per cm^2; rocks well covered
C	0·1 to 1·0 per cm^2; up to one-third of rock space covered
F	0·01 to 0·1 per cm^2; individuals never more than 10 cm apart
O	0·0001 to 0·01 per cm^2; few within 10 cm of each other
R	Less than 1 per m^2; only a few found in 30 min searching

For *B. perforatus*, which is a much larger species, the values are:

A	Over 0·1 per cm^2; close groups on most vertical faces, often up to MTL
C	0·01 to 0·1 per cm^2; adjacent groups, not always above LWN
F	Less than 0·01 per cm^2; adjacent in crevices
O	Less than 0·01 per cm^2; rarely adjacent even in crevices
R	Only a few found in 30 min searching

Limpets

A	Over 50 per m^2 or more than 50 % of limpets at certain levels
C	10 to 50 per m^2, 10 to 50 % at certain levels
F	1 to 10 per m^2, 1 to 10 % at certain levels
O	Less than 1 per m^2 on average, less than 1 % of population
R	Only a few found in 30 min searching

Top-shells

A	Exceeding 10 per m^2 generally
C	1–10 per m^2, sometimes very locally over 10 per m^2
F	Less than 1 per m^2, locally sometimes more
O	Always less than 1 per m^2
R	Only one or two found in 30 min searching

Periwinkles

For the smaller species *Littorina neritoides*, and the small *L. saxatilis* found among barnacles, the ratings are:

A	Over 1·0 per cm^2 at HW, extending down the midlittoral zone
C	0·1 to 1·0 per cm^2, mainly in the supralittoral fringe
F	Less than 0·1 per cm^2, in crevices
R	Only a few found in 30 min searching

For the larger littorinids the ratings are:

A	More than 50 per m^2
C	10 to 50 per m^2
F	1 to 10 per m^2
R	Only a few found in 30 min searching

The other animals, and the plants, are recorded only as present (+) or absent (N), though there may be a differentiation between forms in different habitats.

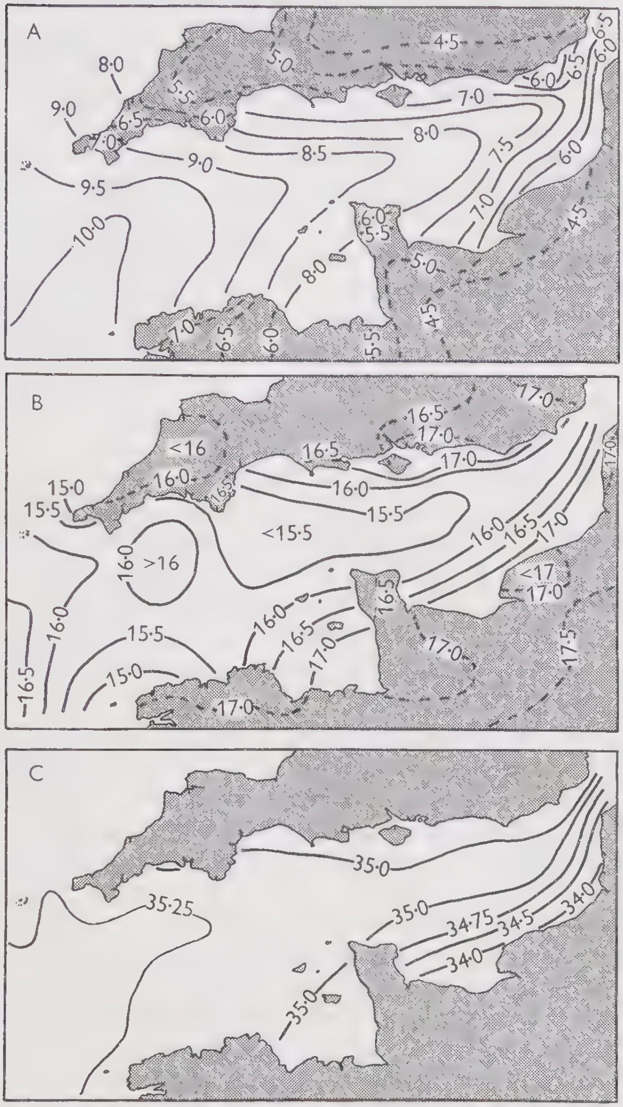
A
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Fig. 1. Some environmental factors in the Channel. A, mean sea and air temperatures for February; B, mean sea and air temperatures for August; C, mean salinity, year. Sea temperatures and salinities are taken from Lumby (1935) and represent conditions pertaining over 30 years ago. Air temperatures have been abstracted from Air Ministry (1936) and Office Nationale Météorologique (1940, 1942*a*, *b*) and isotherms drawn to show actual temperatures not corrected to sea-level; again conditions pertaining some time ago (at least 20 years) are shown.

There may be a sharp discontinuity of the isotherms over the intertidal zone (cf. Fig. 1 A), and neither figure represents the actual temperature of the intertidal zone (Southward, 1958). However, the trends of both sea and air temperatures are similar, at least in the Channel, and it can be assumed that similar trends occur intertidally.

In winter, both sea and air temperatures are influenced by the flow of water eastwards through the Channel. There is a marked temperature gradient from the offshore water, inshore and over the land. In general the temperatures of the inshore water and the coastal air temperatures are some 2–3° C higher in the western half of the Channel than the eastern half. There is, in addition, a further gradient in a north-south direction, for the sea off Brittany is about 0·5° C warmer than the coastal waters of Devon and Cornwall. The coastal air is slightly colder in Flanders and east Normandy than in Sussex and south Kent. This means that there is a steeper east–west gradient of temperature along the French coast than along the English coast.

In summer there is less difference between the east and west of the Channel, the sea and air temperatures ranging from 15 to 17° C. The greatest difference is shown by the relation of the offshore and inshore sea temperatures. In the western half there is still an inflow of warm water from the ocean, and the inshore temperatures may be 0·5–1·0° C cooler, especially in Brittany: in the eastern part of the Channel the inshore waters are generally 1·0–1·5° C warmer than the eastward flowing offshore water, while in estuaries and shallow bays even greater local warming can occur.

Air temperature in summer seems to be related to the topography of the coastline. Higher temperatures are found on the eastern side of headlands and peninsulas, e.g. Start Pt. and Cherbourg. On the other hand, certain exposed coastal areas facing west, such as the north Cornish coast, west Cherbourg and the Le Havre area tend to be cooler than other neighbouring regions.

Salinity

The salinity of the surface water of the Channel shows both an east–west gradient and an inshore–offshore gradient as the high salinity water entering from the ocean becomes diluted by run-off from the land (Fig. 1 C). The differences are greatest in the winter, when more rain falls, but are probably of little importance to most shore organisms, which experience larger local changes in salinity from the direct effect of rainfall and streams on the shore.

Nutrient salts

As far as can be ascertained there are no great differences in the phosphate and nitrate resources of the main body of water in different parts of the Channel proper. Locally rich patches of lower salinity and high silicate content, however, occur in the vicinity of estuaries, while richer high salinity water may be found off the Channel mouth (Harvey, 1930; Kalle, 1937).

Tides, currents and water movements

If we divide the Channel by a line running from Swanage to Cherbourg we find that in the two basins thus formed the tides are out of phase. In the western basin the maximum spring tides occur at midday and midnight, while in the eastern basin they occur in the early morning and evening. Fast tidal currents are found where the two basins join, especially off headlands such as St Catherine's Pt., The Needles, St Alban's Head, Portland Bill, Cap de la Hague and Alderney. The greatest and smallest tides of the Channel are found in this middle section also. The least tides occur on the English side, where the shallow water effects are large and produce the 'double' or 'abnormal' tides of Portland, Swanage and the Isle of Wight. On the French side very big tides occur in the Gulf of St Malo.

The main residual current is up-Channel, the direction of the prevailing winds. Water enters at the western end and passes eastwards through the Straits of Dover. Carruthers (1930) has described the routes taken by drift bottles liberated in the western Channel, while more recently measurements of the total flow through the Straits of Dover have been made by Bowden (1956). It need scarcely be pointed out that the rate of exchange of water with the Atlantic is much greater in the western section of the Channel than it is in the eastern part. Little is known of the minor eddies and counter currents which may exist near the coast and which may be of greater importance in dissemination of larval stages of many intertidal species. A counter eddy has been established in Pevensey Bay from current measurements made at the Varne lightship (Carruthers, 1935; Carruthers, Lawford & Veley, 1950). Counter currents are also indicated on either side of the main drift through the Straits of Dover by Van Veen (1936). It is possible that a counter current sometimes flows eastwards along the south coast of Cornwall and flows clockwise round the south-west peninsula towards the Bristol Channel, but current measurements at the Seven Stones Light Vessel, between Scilly and Land's End, show a residual drift in the direction E.S.E. (Carruthers, Lawford, Veley & Gruning, 1951).

On theoretical grounds the water movements up-Channel would be expected to be deflected southwards very slightly by Corioli's force, while those down-Channel would be deflected northwards. Thus the main residual flow from west to east would have a tendency to hug the French coast, while counter currents might be expected to develop more readily on the English side. However, Corioli's force influences mainly the movement of large water masses which are moving freely with their own inertia. The surface layers of water, and especially those close to the shore where friction with the sea bed is important, however, should tend to follow the direction most closely approximating to that of the wind consistent with the lie of the shoreline.

Wave-action and substratum

The effects of wave-action on the plants and animals of the shore are modified by the type and configuration of the shoreline and the depth of water offshore, and these factors are interrelated. This fact does not seem to have been realized by previous workers, who assessed wave-action on the basis of topography or orientation to prevailing winds (Moore, 1935; Lysaght, 1941). Over small areas of coastline it is sometimes possible to assess wave-action by relating a series of measurements of wave wash above normal tidal height with winds of a certain direction and strength (Southward, 1953), but this method fails on coasts open to ocean swell (Southward & Orton, 1954).

On gently shelving rocky ledges wave wash may surge great distances up the shore, while on very broken rocky reefs splash may be the main effect. The mechanical action of waves also depends upon the nature of the shore; on steep slopes there will be great dynamic shock, while on shelving shore there will be mainly a tearing or shearing effect. Moreover, the presence of submerged reefs or bars offshore may reduce wave-action on the shore or may modify the type of wave reaching the shore (Bigelow & Edmonson, 1947).

We are thus as far as ever from the attainment of some measure of wave-action, since so much depends on local factors. Local factors are even more important on depositing shores, or where large amounts of mud, sand or gravel are found offshore. These particles are moved or brought into suspension, according to their size and the power of the waves, and can scour the rocky parts of the coast. In general, the greater the wave-action at a place, the larger the particles remaining to be moved or affected by wave-action, for the smaller particles have been removed (cf. de Martonne, 1935). Thus headlands jutting out into deep water, though they receive greater pounding by waves, generally show little scour, while bays with accumulations of sand or gravel, though often receiving less wave-action, may be exposed to greater damage by scour. The scouring action of gravel seems to be especially severe, and predominantly gravel shores are virtually intertidal deserts.

The western half of the Channel is bounded by a steep rocky shoreline indented at intervals by drowned valleys and sandy bayhead beaches, and deep water is never far offshore. Largely because its shores consist of softer material the eastern half of the Channel has a more even coastline, and the great accumulations of sand and shingle form gently shelving beaches. The eastern basin is shallow and wave-induced movements must often disturb the sea bed. Thus in the eastern Channel the water carries much suspended matter, and near the shores the waves cause continual abrasion through the sand and shingle that they churn up. This abrasion is most marked near high-water mark where the largest stones are found and where the waves break with greatest force. In the western basin, however, the water is generally clearer, and carries little particulate material; the shores are wave-beaten rather than scoured. This generalization is true only in so far as these shores are of harder rock; where the rock is more friable, or where much material is brought down by rivers, coastal conditions approximate to those of the eastern end of the Channel. Thus the coasts surrounding the Helford River, the Fal and Fowey, Plymouth Sound, Salcombe and the Dart are locally silty and muddy with turbid water at spring tides and after rain. Mounts Bay is extensively sand-scoured, while much of Lyme Bay has accumulations of shingle, culminating in the famous Chesil Bank. The scour experienced by many of the coasts lying between the Exe and Chesil Bank must surely equal or exceed that suffered anywhere to the east of Portland. Nevertheless, the Channel water as a whole remains very clear throughout most of its length as far as the Isle of Purbeck. Only where the Channel narrows and shallows to the east of the Isle of Wight does the water near the shore become almost continuously turbid.

Similar conditions apply to the French coast. The long estuaries of Finistère and the Côtes du Nord produce locally turbid conditions, and the scour is probably greater than in corresponding drowned valleys on the English side, on account of the greater tidal range. The Gulf of St Malo, particularly towards its south-eastern corner, is in some respects analogous to West Bay. Its coasts have been eroded to produce much sand, gravel and shingle, which is prevented from being driven eastwards by the Cherbourg peninsula. Hence this loose material has accumulated both in the bay, reducing its depth, and on the west-facing shores south of Carteret where the scour appears to be severe.

Two small but important differences between the French and English coasts should be referred to here. First, at the extreme western end of the Channel the coast of north Finistère is less steep than that of south Devon and Cornwall, and is protected by numerous offshore reefs and islands. Moreover, the predominant wind at the mouth of the Channel, which is south of west, blows almost directly on to the coast of south-west England, while northern Finistère lies slightly in the lee of the Armoricaine peninsula. Hence a considerable part of the latter coast, excluding the offshore islands, is less exposed to wave-action; in general, it retains more loose material in the shape of sandy beaches and boulder spits (e.g. Sillon de Talbert) than corresponding regions of the English coast. Secondly, at the extreme eastern end of the Channel, the English side is composed entirely of soft chalks, sands and alluvium, yielding intertidally only chalk reefs and sand or shingle beaches. On the Boulonnais coast, however, exposures of oolite face the prevailing winds and give rise to more typical rocky shores below H.W.N., even though banks of shingle may form at high-water mark.

It is worth pointing out that geological factors profoundly influence the formation and character of tide pools, which are the main habitat of some animals and plants. In the eastern part of the Channel the rocks are usually too friable or too flat-bedded to allow the formation of other than shallow pools, which are often filled with sand or other deposits.

The main differences between the two halves of the Channel are summarized in Table 3.

TABLE 3. MAIN ENVIRONMENTAL DIFFERENCES BETWEEN THE WESTERN AND EASTERN PARTS OF THE CHANNEL

Factor	West	East
Summer temperature	Little lower	Little higher
Winter temperature	Higher	Lower
Average temperature	Higher	Lower
Tendency to frosts	Slight	Considerable
Average sea salinity	Higher	Lower
Nutrient salts	Little difference	Little difference
Biological factors	Decreasing from west to east?	Not known
Tidal currents	Less	More
	(very great in middle)	
Scour	Little	Much
Silt in suspension	Little	Much
Rocky substratum	Much	Less
Pollution	Some	More
Wave-action	More	Less, but bottom disturbance greater
Depth of water	More	Less
Thermocline in summer	Probably stable	Probably disturbed in shallower water by currents

THE DISTRIBUTIONS

The main trends in distribution of the organisms investigated have already been summarized in Table 1. The detailed distribution of the commoner animals is set out in Table 7 (appendix). We have not tabulated in detail our results for the algae, as these were not as completely investigated as the animals on the English side of the Channel. The algal distributions, and those of some of the less common animals also investigated in less detail, are commented upon below, after the commoner animals.

COMMONER ANIMALS

Anemonia sulcata

The records for *Anemonia sulcata* in Table 7 refer mainly to positive occurrences, as the species may be found only at extreme low water and its complete absence from a locality cannot always be stated with certainty. The records refer to both ecological forms of the species, the one in pools in the upper midlittoral and the other among algae in the infralittoral fringe. Both forms are common in the western part of the Channel, but only the low-water form occurs towards the east. The species penetrates sporadically into the eastern basin, the most easterly records being at Brighton and Grandcamp.

Actinia equina

The commoner of the two species of sea-anemone investigated, *Actinia equina*, occurs throughout the Channel. Fischer-Piette (1936) regarded the distribution of the species as 'capricious', and his charts show areas of greater or lesser abundance. Our results differ: we found the species common at some localities where he did not find it, for example Tremazan and Cancale, and no general trend in distribution could be made out, save that the greater area of suitable rocky substratum at the western end of the Channel resulted in the species being found at a greater proportion of the stations than in the eastern basin.

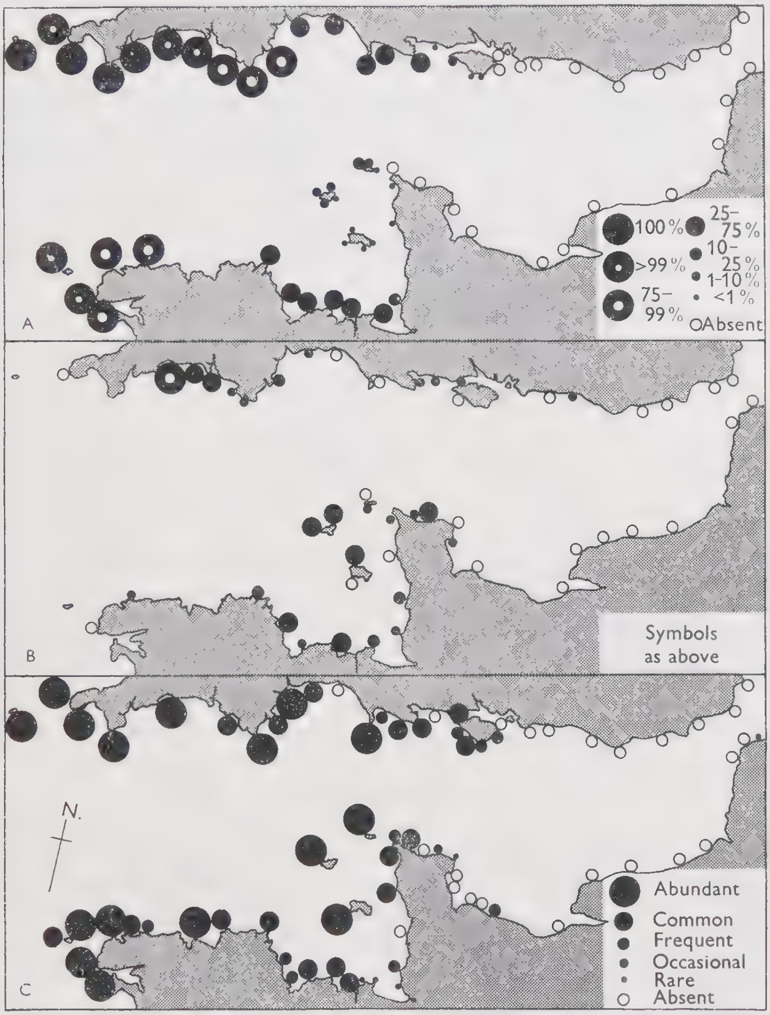
100 %
25–75 %
>99 %
10–25 %
1–10 %
75–99 %
<1 %
Absent
A
B
Symbols as above
N.
C
Abundant
Common
Frequent
Occasional
Rare
Absent

Fig. 2. Distribution of some representative species in the Channel. A, confined exclusively to the western part; *Chthamalus stellatus*, shown as percentage of population of *Chthamalus* and *Balanus balanoides*; B, common in the west and rare in the east; *Hemioniscus balani*, shown as percentage infection of *Balanus balanoides*; C, present in the eastern basin on the French side only; *Littorina neritoides*.

Possibly the abundance of *Actinia* varies greatly from year to year, or over quite small distances on the shore. No doubt the viviparous habit of breeding would contribute to such variation.

Chthamalus stellatus

The barnacle *Chthamalus stellatus* is confined to the western half of the Channel, where its abundance increases westwards, more or less inversely with the abundance of *Balanus balanoides*. It is more abundant on the English side of the Channel, and extends farther east there than on the French coast. The difference between the two coasts is clearly brought out if we tabulate the mean abundance at topographically comparable stations or compare the relative proportions of the two common species of barnacles (Table 4, Fig. 2).

The distribution and abundance of *Chthamalus* in 1949–57, as shown by our observations, are very different from those in the 1930's, reported by Fischer-Piette (1932, 1936) and Moore & Kitching (1939). The changes on the English coast reported in previous publications (Southward & Crisp, 1954*a*, 1956) seem to have been accompanied by similar, but less marked changes on the French side, notably in the Gulf of St Malo (Table 5). There seems to have been a slight increase in range of the species north of Carteret and to the north-east of Alderney, and at the same time a marked increase in density at all but three of the stations where the species was present in the 1930's. The increase appears to have been most marked in the outermost Channel Islands, Alderney and Guernsey, where *Chthamalus* is now present in greater numbers than in Jersey, a reversal of the previous situation.

TABLE 4. MEAN BARNACLE COUNTS TAKEN AT THREE TIDE-LEVELS AT CORRESPONDING STATIONS ON THE COASTS OF ENGLAND AND FRANCE*

Numbers per square centimetre of rock surface.

		Balanus balanoides		*Chthamalus stellatus*	
English station	French station	England	France	England	France
Cape Cornwall	Le Conquet	0·002	0·012	3·0	3·8
Sennen Cove	Tremazan	0·02	0·02	2·8	2·3
Lizard Head	Pt. du Pontusval	Nil	0·001	4·7	2·0
Polkerris	Primel-Trégastel	0·0001	0·001	3·2	3·0
Hope Cove	Sillon de Talbert	0·03	1·3	3·4	1·4
Brixham	St Quay-Portrieux	0·75	2·5	2·8	2·1
Dawlish	Pt. du Roselier	2·3	1·3	3·5	2·6
Budleigh Salterton	Pt. de Pléneuf	3·0	1·4	2·5	4·0
West Bay	Dinard	0·9	2·1	2·6	1·9
Portland Bill	Cap de la Hague	1·0	> 1·0	2·9	Nil
Peveril Pt.	Cap Lévy	0·11	> 1·0	0·12	Nil
St Catherine's Pt.	Pt. de Barfleur	0·5	> 1·0	0·001	Nil
Bembridge	Pt. de Hoc	4·1	> 1·0	Nil	Nil

* For dates and further details see Table 7 (appendix).

TABLE 5. CHANGES IN THE ABUNDANCE OF *CHTHAMALUS STELLATUS* AT STATIONS IN THE GULF OF ST MALO

As maximum density per square centimetre, or ratio to *Balanus balanoides*. Brackets indicate nearby stations, to be compared with each other.

	1930–32 (from Fischer-Piette, 1932, 1936)	1954–6
Cap de la Hague	Absent	Absent
Nez de Jobourg	Absent	—
Vauville	—	0·0005
Flamanville	Absent	—
Carteret	0·01	0·01
Granville	1:20 at H.W.N.	3:4 at H.W.N.
Clonque	0·0005	0·5
Chateau a l'Etoc	Absent	—
Pt. Quesnard	—	0·004
La Moye Pt.	0·006	0·36
Fort Doyle	0·0001	0·01
Montorgueil Castle	Very rare, just above upper limit *B. balanoides*	0·01
Plemont Pt.	Very rare, just above upper limit *B. balanoides*	0·04
La Corbière	Very rare, just above upper limit *B. balanoides*	0·015
Cancale	1:20 at M.T.L.	2:1 at M.T.L.
Dinard	—	5:4 at M.T.L.
Pt. du Decollé	1:1 at M.T.L.	—
Pt. de Pléneuf	1:1 at M.T.L.	2:1 at M.T.L.
Pt. de Pordic	3:1 at M.T.L.	4:5 at M.T.L.
Sillon de Talbert	2:3 at M.T.L.	1:3 at M.T.L.

Balanus balanoides

The common acorn barnacle *Balanus balanoides* is present throughout the Channel. Our records agree generally with those of Fischer-Piette (1932, 1936; personal communications) except for south-west England where the species is less abundant than formerly. During 1949–57 *B. balanoides* was rare west of Plymouth and was absent from the Isles of Scilly and the extreme south-west of Cornwall (Figs. 2, 6). The species was more common on the French coast opposite, and was found without difficulty at most stations examined between Roscoff and the Rade de Brest, though frequently confined to low water and never very common. Comparison of representative stations on both sides of the Channel (Table 4) shows that the species is generally more abundant on the French side. Only at two pairs of stations is the incidence of *B. balanoides* less on the French side. The French stations in these cases, Pt. du Roselier and Pt. de Pléneuf, are relatively exposed headlands, while the corresponding English stations do not stand out from the coastline.

On both coasts of the western basin the abundance of *B. balanoides* increases towards the east, but not uniformly. There is a region of reduced density on the English coast, from West Bay to St Catherine's Pt., that is not paralleled by a corresponding reduction on the opposite coast. As *Chthamalus stellatus* is also reduced in abundance at many of the stations in the same area, the reduction cannot well be attributed to effects of temperature or wave-action. It seems possible that the small tidal range in the region may reduce the stocks of adults to a point where there is difficulty in producing sufficient larvae for more abundant settlement. The strong tidal currents may also be detrimental to settlement of larvae and may hinder recruitment from more distant stocks of adults.

Our observations support and extend previous conclusions as to the influence of habitat on the abundance of barnacles in the Channel (Fischer-Piette, 1936; Southward & Crisp, 1954*a*, 1956). In the western basin *B. balanoides* thrives better and grows to a larger size in the estuaries and harbours than on the open coast. In the eastern part of the Channel it is dominant on the open coast, and in sheltered bays and harbours is replaced by *Eliminius modestus*. On shores composed largely of chalk rocks, as for example at Lulworth Cove, Rottingdean, North Foreland, Cap Blanc Nez and Fécamp, *B. balanoides*, in common with other species of barnacles present, was reduced in number and occurred only sporadically or in sparsely scattered groups. Similar low barnacle counts were found on other soft rocks, such as the Wealden clays exposed at Brook and Fairlight. The relative paucity of barnacles on soft substrata has been commented on previously (Southward & Crisp, 1954*b*), and has been attributed to porosity, with consequent desiccation through the basis (Moore & Kitching, 1939) or to the enhanced erosive activities of limpets on such rocks (Hawkshaw, 1878). The poor adhesion of the base on soft rocks must be a contributory factor, together with the scouring effects of the loose flints that are almost always present on chalk shores, and all these factors probably operate together on the open coast. In sheltered waters, however, where there is little wave-action, where limpets are less abundant and where loose substrata do not move, barnacles may be found in good number on soft materials.

Balanus perforatus

Like *Chthamalus*, *Balanus perforatus* is confined to the western half of the Channel, and is not found east of the Isle of Wight or Cotentin. *B. perforatus*, in recent years, seems also to have undergone an increase in abundance, which may have begun in the 1930's or earlier, for Fischer-Piette (1936) remarked on an increase in the Channel Islands between 1930 and 1935. No doubt, as a southern species, *B. perforatus* might be expected to benefit from any general increase in temperature, such as occurred in the Channel in recent years (Southward & Crisp, 1954*a*; Cooper, 1958). It breeds and settles in a limited period of the late summer and autumn (Norris & Crisp, 1953), and would receive most benefit from the warm autumns that have been a marked feature of the climatic amelioration. Nevertheless, the evidence for increased abundance of this species must be viewed with caution. It is a barnacle that may be surprisingly locally distributed, and one that is profoundly influenced by wave-action and the configuration of the shore. Observations of its abundance may be very subjective, and we ourselves have noted considerable differences in our individual assessments of its abundance at a station. For these reasons it is difficult to compare our results with those of Fischer-Piette (1936), though we have tried to do so (Table 6). In considering the earlier observations, it is hard to believe that in 1932 *B. perforatus* was absent from Wembury (it was recorded by Colman, 1933) or the Isles of Scilly, though the abundance may have been less than it is now. This throws some doubt on the apparent changes that have occurred at the other English stations.

TABLE 6. RELATIVE ABUNDANCE OF *BALANUS PERFORATUS* AT STATIONS ON THE ENGLISH COAST

	1930–2 (from Fischer-Piette, 1936)	1949–57
St Catherine's Pt.	N	O
Portland Bill	O–R	A
Straight Pt.	A	—
Budleigh Salterton	—	A
Brixham	C	C
Prawle	O–R	A
Wembury ('Blackstone Pt.')	N	A
Rame Hd.	C	A
Lizard Hd.	O–R	C
Lamorna Cove ('Carn du Pt.')	C	A
Cape Cornwall	O–R	C
Isles of Scilly	N	F–A

These terms of abundance are explained in Table 2. We have converted Fischer-Piette's terms as follows: Grande abondance, A; Abondance moyenne, C; Faible, F; Très faible, O–R.

We can, however, be certain that Fischer-Piette's evidence that *B. perforatus* was commoner on the French side of the Channel no longer holds good. In fact, at the western extremity, the species is commoner on the English side. Notable changes seem to have taken place in the Channel Islands, as with *Chthamalus stellatus*. Thus, in Alderney and Guernsey, where *Balanus perforatus* was reported 'très faible' or absent in 1931, we found it to be quite common at most exposed places and extremely abundant on the outer edges of the reefs. In Jersey, on the other hand, the species appeared to be rather uncommon, and only in the north-west of the island did we find it in numbers approaching those present on the outer two islands. We did not see the species at two stations where it was reported previously to be 'faible' or 'très faible', probably because our visit did not allow each station to be worked on a very low tide.

The revised distributions of *B. perforatus* and *Chthamalus* in the Gulf of St Malo agree well with one another (Fig. 5, p. 184). Both are relatively common on the coasts of Ille-et-Vilaine and Côtes-du-Nord, and become scarce along the western coast of Cotentin. They are both rather uncommon in

Jersey, and more abundant in Alderney and Guernsey, although *Chthamalus* is not there as abundant as on the coasts of Ille-et-Vilaine and Côtes-du-Nord. If we compare the extreme limits of *Balanus perforatus* in 1930–32 and 1949–57, there would seem to have been an extension of range eastwards along the English coast, corresponding to the extension of *Chthamalus* during the same period. Fischer-Piette (1936) did not find the species east of St Alban's Head: it is now common in Poole Harbour (Norris & Crisp, 1953), and can be found without difficulty in parts of south Hampshire and the Isle of Wight. Dr H. G. Stubbings informs us that occasionally he has found spat of *Balanus perforatus* on settlement panels exposed at the entrance to Chichester harbour, but so far we have been unable to find any adult specimens in Sussex.

B. perforatus is sometimes locally much commoner in bays and fairly saline estuaries than on the open coast as, for example, in the Halford and Fal estuaries as compared with the Lizard peninsula (Fig. 6, p. 191). However, it can be very abundant in some exposed situations, and there is no general rule that the species is commonest in embayed situations, as might be inferred from Fischer-Piette's evidence. The species is extremely abundant on the Eddystone rocks, off Plymouth, and on the outermost blocks of the Plymouth breakwater, both isolated sites where wave-action is severe and prolonged. The species is also common on sand-scoured reefs in Mounts Bay and Whitsand Bay. The action of the waves, whether accompanied by scouring effects or not, does not therefore seem to be directly inimical to the species.

Balanus crenatus

We have not been able to investigate *Balanus crenatus* in sufficient detail because, on the open coast, it is often present only at extreme low-water mark. Our records, as far as they go, indicate that the species is generally distributed along the Channel, though possibly more in evidence at the eastern end. In the western half of the Channel *B. crenatus* resembles *B. balanoides* and *Elminius* in growing better in estuaries and harbours than on the open coast. Specimens from the Dart, Plym, Penzé and l'Aber Beniot estuaries commonly reach 15–20 mm in diameter, and may occur up to M.T.L., compared with sizes of 6–9 mm at extreme low water on the open coast.

This species, of course, is very common in the infralittoral zone and may be very abundant on the shells of molluscs and crustaceans dredged from shallow water.

Balanus improvisus

We have included the estuarine species *Balanus improvisus* in our records for comparison with *Elminius modestus*, which is replacing it in many of its habitats. *Balanus improvisus* was present throughout the Channel in the estuaries, from the Helford and l'Aber Beniot in the west, to the Rother and Boulogne in the east.

Elminius modestus

Detailed accounts of the spread of the immigrant Australasian barnacle *Elminius modestus* along the Channel coasts will be found elsewhere (den Hartog, 1956; Crisp, 1958; Bishop & Crisp, 1957, 1958). During the later part of the survey the species was abundant in the eastern part of the Channel, but common only in the estuaries and harbours in the west. On both sides of the Channel there was, in 1954–56, an intermediate region in which *Elminius* had not established itself. On the English side it was absent from the greater part of Lyme Bay: on the French side the uncolonized area was larger, and included the whole of the Gulf of St Malo and the Channel Islands.

Verruca stroemia

We have attempted to study the distribution of the low-water species *Verruca stroemia* in more detail than the other species found only at the lower levels of the shore, since its general European distribution is intermediate between the distributions of the typically northern or southern species. *Verruca* is most commonly found in shallow water below the tide marks, but may be quite abundant in the infralittoral fringe in places. Its most favoured habitat is on the undersides of rocks and stones where there is little movement of the substrate, but where there is enough water movement to prevent the deposition of large amounts of silt. Incomplete observations on *Verruca* suggest that it may be restricted by its feeding habits to places with some water currents, for it does not always set up a current by rapid beating of the cirri as other barnacles do (cf. Southward, 1955*c*).

Where conditions are satisfactory the species can be very abundant in the intertidal zone, as for example at Brixham, as noted by Fischer-Piette (1936), on the stones and boulders of the breakwater. At Plymouth it can be found in widely distinct habitats such as the outer reefs at Wembury, and the fairly silty conditions at Hen Pt. in the Tamar. *Verruca* is common on most shores at the western end of the Channel, but seems to become sparser eastwards. The most easterly record, where two or three specimens only were found, is Fairlight, Sussex. The relatively scanty records may perhaps be filled in by use of planktonic occurrences of the larvae, which are quite distinctive (Bassindale, 1936), but this method inevitably takes account of infralittoral populations also. It seems probable that the species is absent from the southern North Sea, for larvae have not been seen in inshore plankton samples from Essex. The absence of *Verruca* nauplii from hauls at Brighton confirms that the adult cannot be very common in the eastern half of the Channel. At Brixham, Plymouth, and farther west in the Channel (see p. 193) *Verruca* is often the most abundant cirripede larva in the plankton in spring.

Hemioniscus balani

We became interested in the parasitic isopod *Hemioniscus balani* since there appeared to be some relation between its distribution and abundance and the abundance of its common host, *Balanus balanoides*. The latter is not, of course, the only host in the Channel since *Hemioniscus* has been found in *Elminius* and *Balanus improvisus* as well as in *B. balanus* (Perez, 1923; Crisp & Molesworth, 1951). Our records relate to the larger gravid female stage only, as the smaller male stages are often overlooked in the field.

It has already been shown (Southward & Crisp, 1954*a*) that the incidence of the parasite in the western part of the English side of the Channel in 1949–52 was much higher than farther east; the later observations confirm and extend these results. The species was found almost everywhere in the western basin, often at very high percentages of infection, but was very sparse or absent altogether in the eastern basin where the host was more abundant.

Since *Hemioniscus* occurs in the North Sea, some explanation other than the influence of climate alone must be found for its relative sparsity in the eastern part of the Channel. It is perhaps significant that there was an apparent increase in the rate of infection by *Hemioniscus* in Devon and Cornwall between 1949 and 1952 (Southward & Crisp, 1954*a*), while the host *Balanus balanoides* decreased in abundance. More recent observations show a smaller percentage of infection in the following years, coincident with a general resurgence of the host (Southward & Crisp, 1956).

Just as a change in average temperature may alter the balance between competing species such as *Chthamalus stellatus* and *Balanus balanoides*, it may influence a host-parasite relationship. On this theory we have only to postulate that *Hemioniscus* passes through its life cycle more quickly at higher temperatures, *Balanus balanoides* being limited in its reproduction to a single annual cycle. Further work is clearly necessary before a definite conclusion can be reached on the dynamics of the relationship between *Hemioniscus* and its hosts.

Patella vulgata

The common limpet, *Patella vulgata*, was found on all solid substrata throughout the channel. On the English side it was the only limpet present east of the Isle of Wight, as reported by Evans (1953).

Patella depressa

Patella depressa (= *P. intermedia*) is confined to the western half of the Channel. The eastern limits are at Ventnor and Culver Cliff (Fischer-Piette, 1936) on the English side, and Barfleur on the French side. At these stations the range of the species ends abruptly, without undergoing any gradual diminution in abundance such as occurs with other animals. At most wave-beaten localities in the western basin it constitutes from 10 to 50%, or frequently more, of the limpet population around mid-tide level. It is scarce or absent on part of the west coast of Cotentin, where the rocks seem to be made unsuitable for limpets by sand scour. On both sides of the Channel it is found in less abundance at the extreme western end. It is absent from the Isles of Scilly, rare in Ushant (Fischer-Piette, 1936), and the abundance on the open coast does not exceed 50% at any place west of Plymouth. In Finistère our records differ from those of Fischer-Piette (1936) in showing that the species is not entirely absent from Tremazan. A few specimens were found in a limited region towards low water, but the shore appeared generally unsuitable to limpets and barnacles, possibly because of the dense algal growths. At other open coast stations farther east *P. depressa* constituted more than half the limpet population at some levels.

On extremely wave-beaten rocks the limpet population tends to be dominated by *P. aspera*, which seems more tolerant of excessive wave-action or wetting. For example, on the Eddystone rocks off Plymouth the larger limpets are almost all *P. aspera*; *P. depressa* has not been found and *P. vulgata* is represented only by smaller specimens towards the upper midlittoral. At Shipman Head, Bryher, Scilly, over 90% of the limpets below H.W.N. were *P. aspera*. These effects of wave-action may explain the reduced numbers of *P. depressa* at some of the more western stations, but larval dispersal may also be involved (see p. 194).

Patella aspera

The distribution of *Patella aspera* corresponds closely with that of *P. depressa*, and there is a similar sharp cut-off at the identical stations which mark the eastern limits of the species. *P. aspera* is abundant at most exposed sites in the western basin. We could not detect any general trends in distribution except that the species was scarce on the coast of Cotentin where sand scour is severe.

It must be noted that the agreement in distribution of *P. depressa* and *P. aspera* does not hold good outside the Channel. *P. aspera* extends up the west coast of Britain, and penetrates southward into the North Sea on the east coast of England (evidence to be published later), while *P. depressa* has its northern limit in Anglesey (Crisp & Knight-Jones, 1955) and is absent from Ireland (Southward & Crisp, 1954*b*).

Monodonta lineata

Monodonta lineata is the most conspicuous of the top-shells, since it is common at the upper limit of the midlittoral zone and can be found in the open on bare rock. Like the other top-shells, however, the greatest numbers are found on partly sheltered shores of boulders or broken rock, and it is less common on steep slopes (see Southward & Crisp, 1954*b*). *Monodonta* is abundant throughout most of the western half of the Channel, but extends considerably farther east on the French coast. On the English side the limit is at Lyme Regis; on the French side it is present in small numbers east of the Cotentin peninsula, but is absent from most of the Normandy coast. The boundaries of this species imply that it is limited by factors other than the

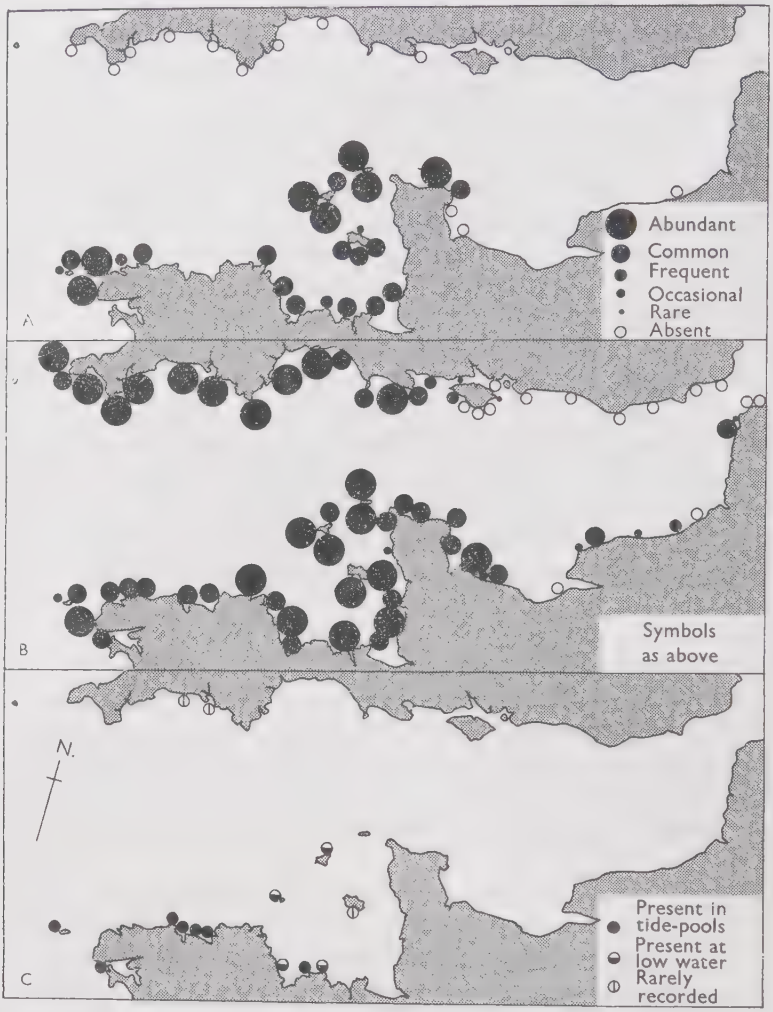
Abundant
Common
Frequent
Occasional
Rare
Absent
A
B
Symbols as above
N.
C
Present in tide-pools
Present at low water
Rarely recorded

Fig. 3. Distribution of some representative species in the Channel. A, confined exclusively to the French side of the western basin; *Gibbula pennanti*; B, quite common along the whole French coast, but confined to the western basin on the English side; *G. umbilicalis*; C, confined to the western basin, but not at all common in the Channel, and rarely found on the English side; *Paracentrotus lividus*.

configuration of the shore, for many apparently suitable shores are found to the east of its range. For example the Dorset coast between Weymouth and Swanage, and the Normandy coast east of Arromanches, both offer very suitable habitats for top-shells if the abundance there of *Gibbula umbilicalis* is taken as a guide. We have unsuccessfully searched the Dorset area several times, since there are old records (Mansell-Pleydell, 1898) of the occurrence of *Monodonta* there. It seems probable that the records are based on casual findings of dead shells; the finding of a 'recently vacated' dead shell at Whitstable, Kent (el Maghraby & Perkins, 1956) shows how misleading such records may be.

Gibbula umbilicalis

The distribution of *Gibbula umbilicalis* follows the pattern of that of *Monodonta*, and although it penetrates somewhat farther east than the latter on both sides of the Channel, there is again a considerable difference between the English and French sides (Fig. 3, p. 176). The most easterly occurrence of *Gibbula umbilicalis* on the English coast is at Bembridge, while on the French side it occurs as far as Calais. The areas where the species was not found on the French coast, around Le Havre and Le Tréport, are rather unfavourable to top-shells, the few rocks being separated by wide stretches of sandy beach. On the English coast the absence east of the Isle of Wight cannot be attributed entirely to the absence of suitable shores, for there are apparently suitable habitats at Seaford, Brighton and near Beachy Head.

Gibbula pennanti

The validity of the species *Gibbula pennanti*, noted as an unnamed variety of *G. umbilicalis* by Forbes & Hanley (1853), has been established by Gaillard (1954). We ourselves did not learn the species until the later part of the survey. For this reason our maps are not as complete as could be desired (Fig. 3A). The adults can be separated easily from *G. umbilicalis* by the closure of the umbilicus and, once learnt, the two are usually distinguishable on shell pattern alone. The uninitiated would be easily misled, for although *G. pennanti* may be commoner at low water in some places, in localities where it is abundant it tends to occur over most of the midlittoral zone and may replace *G. umbilicalis* as the most abundant top-shell. Unlike *G. umbilicalis*, however, *G. pennanti* is confined to the French side of the western half of the Channel. It was found at most stations on the Cotentin peninsula, the Gulf of St Malo, and the Brittany coast, and was common in the Channel Islands, especially in Alderney and Guernsey, but was absent east of Barfleur. Careful searches in the south-west of England, in places where the habitat appeared to be essentially similar to that in which the species is found in France, have failed to disclose a single specimen.

Gibbula cineraria

The top-shell commonly found at low water, *Gibbula cineraria*, was found throughout the Channel. Although it favours broken rocky shores like other top-shells, the species is much less sensitive to the presence of sand, and may sometimes be common in predominantly sandy places. Unlike the other species of *Gibbula* discussed above, it is quite common infralittorally, especially among algae.

Littorina species

The common small periwinkle, *Littorina saxatilis*, is abundant throughout the Channel. It seems very tolerant of scouring and of low salinity. *L. obtusata* is also common everywhere where fucoids are plentiful.

The edible winkle, *L. littorea*, is present throughout the Channel on the more sheltered shores. It is intolerant of wave-action, and is scarce at the western extremities of both sides of the Channel, and very rare in the outer Channel Islands. We did not find it in the Isles of Scilly, but it was recorded there by Clark (1906), and Mr G. M. Spooner tells us that he found two specimens in 1936.

L. neritoides is abundant on exposed shores throughout the western half of the Channel. On the English side it is present in the Isle of Wight, where the eastern limit is Sandown. On the French coast it penetrates somewhat farther into the eastern basin; it was present in small numbers on the Normandy coast east of Cotentin, and two specimens were collected at Calais.

Paracentrotus lividus

The sea-urchin, *Paracentrotus lividus*, which is such a noticeable feature of intertidal pools on the Atlantic coasts of Ireland and France, is much rarer in the Channel, where it is often confined to extreme low-water mark or the infralittoral zone. For this reason we have few positive records, and have relied on earlier observations to compile Fig. 3c. *Paracentrotus* can be regarded as virtually absent on the English side, specimens having been found only occasionally at Looe and Wembury (Marine Biological Association, 1957; personal recollections of the Plymouth Laboratory staff; preserved material in the faunal collection at Plymouth). On the French side our observations confirm that the species is common only in the neighbourhood of Roscoff (Station Biologique de Roscoff, 1951*a*) where it is present in pools towards low water. It is less common in the Bay of St Brieuc, at the Roches Douvres and in Guernsey (Koehler, 1884; Fischer, 1928). No specimens were found during our survey in the extreme north-west of Brittany, but observations were not made on the more exposed islands offshore where *Paracentrotus* may be more common, as it is in Ushant.

OTHER ANIMALS

Balanus balanus

Balanus balanus (= *B. porcatus*) is not common intertidally, but its distribution has always interested us, as it is a species of markedly northern character. It is present on the shore, at extreme low water, in Anglesey and the Isle of Man, and there are recent records of its occurrence at low water, or just within the infralittoral zone in south-west Ireland (Lilly, Sloane, Bassindale, Ebling & Kitching, 1953). It has been found on test panels from Milford Haven, and has been recorded by Bassindale (1941) in other parts of the Bristol Channel. The species seems to be common in the North Sea, but we have been unable to confirm any of the records of its occurrence in the Channel. It has not been seen on the shore, and has never been found on dredged material brought into Plymouth or Brixham. There exist specimens in the Jeffreys collection, now in the U.S. National Museum, labelled as from Exmouth (Pilsbry, 1916). The authenticity of some of the Jeffreys material is doubtful, however, since the collection includes specimens of *B. perforatus* labelled as from Irish and Scottish localities where the species does not occur (Norris & Crisp, 1953; Southward & Crisp, 1954*b*). The most reasonable view of these discrepancies in the Jeffreys collection is that some of the labels have been exchanged.

At the moment we conclude from the limited evidence that *B. balanus* is absent from the western half of the Channel and scarce or absent in the eastern half.

Haliotis tuberculata

The ormer, *Haliotis tuberculata*, found mainly at extreme low water or infralittorally, is restricted entirely to the western half of the French side of the Channel, where the limits are Alderney and Cherbourg (Wegmann, 1884; Sinel, 1906; Stephenson, 1924; Crofts, 1929; Tomlin, 1937; Station Biologique de Roscoff, 1951*b*). We have few personal records, but local collectors confirmed that the species is still quite abundant in Guernsey and Alderney, where it may be commoner than in Jersey, and in north Brittany.

Gibbula magus

The top-shell *Gibbula magus* is predominantly infralittoral, but is sometimes common at extreme low-water mark in the Channel. Its distribution intertidally appears to follow the trend of the other top-shells of southern character, for the species is much more frequent on the French side. It is common on sandy shores. We found it in some number on the Cotentin peninsula, as far east as Grandcamp, and it appears to be abundant around Roscoff (Station Biologique de Roscoff, 1951*b*). *G. magus* sometimes occurs intertidally near Plymouth (Marine Biological Association, 1957) but numbers approaching those found on the French coast have been encountered only in the Isles of Scilly.

Calliostoma zizyphinum

A further top-shell, *Calliostoma zizyphinum*, has been included in the detailed table (Table 7, Appendix), but the distribution is not clear. Since the species can occur infralittorally only positive records have been noted. *Calliostoma* is present throughout the Channel, and has been recorded from the vicinity of Ramsgate (Woodward, 1880). That more records from the western basin than the eastern basin are included in the table may be due to the greater amount of rocky shores available in the west, and it is not possible to be sure of any trends in distribution.

PLANTS

Only the commoner species of large *Phaeophyceae* could be investigated (Table 1, p. 159). No doubt the other classes of algae have species with corresponding types of distribution (cf. Fischer-Piette, 1936).

Laminaria ochroleuca

Probably the most southern in distribution of the laminarians, *L. ochroleuca* has been recognized in the Channel only in more recent years. There seems good evidence for believing that it has increased its range and abundance in the Channel (Parke, 1948). It is now common in south-west Cornwall and around Plymouth, and extends as far east as the Salcombe estuary (Spooner, 1950), but is apparently absent from Torbay and farther east (personal communications, Mr G. M. Spooner). On the French side the distribution corresponds with that of the southern types of animals. It is abundant at the western end, and has its eastern limit about Alderney and Barfleur, but is apparently absent in the Gulf of St Malo, Jersey, Guernsey and most of Cotentin (Hamel, 1931–39; Lami, 1943, 1954; Station Biologique de Roscoff, 1954).

Laminaria spp.

The three other species of *Laminaria* present in the Channel, *L. digitata*, *L. hyperborea* and *L. saccharina*, occur throughout. *L. digitata* and *L. hyperborea*, which grow best on firm substrata where there is some exposure to wave-action, may be less common in the eastern basin.

Saccorhiza polysichides

The laminarian *Saccorhiza polysichides* is of relatively southern character; like *Laminaria ochroleuca* it seems to be absent from the eastern half of the Channel. On the French side the eastern limit is just north of Barfleur (Hamel, 1931–39).

Alaria esculenta

The only laminarian to favour very wave-beaten sites, *Alaria esculenta*, is found exclusively in the western basin of the Channel. On the French side it occurs as far east as Alderney and Cherbourg (Fischer-Piette, 1936; Hamel, 1931–39). We were unable to visit many of the really exposed sites that the species favours, but have confirmed that it is abundant in Alderney. On the English side *Alaria* does not seem to penetrate as far east. We have found the species as far east as Portland Bill, but it is not common to the east of Start Pt. Its southern limit is just south of the entrance to the Channel (Dizerbo, 1947).

Ascophyllum nodosum

Ascophyllum nodosum is present throughout the Channel, but appears to be commoner in the western half (Hamel, 1931–39), especially in estuaries and sheltered bays.

Fucus and *Pelvetia*

The fucoids *Fucus spiralis*, *F. vesiculosus*, *F. serratus* and *Pelvetia canaliculata* are present throughout the Channel.

Himanthalia elongata

We have confirmed that *Himanthalia elongata* is confined to the western half of the Channel (Fischer-Piette, 1936; Hamel, 1931–39). The eastern limits are Peveril Pt. and Barfleur, but the species is absent from the inner parts of Lyme Bay and the Gulf of St Malo. This distribution has been discussed in some detail by Fischer-Piette (1936).

Bifurcaria rotunda

There is little to add to previous accounts of the distribution of *Bifurcaria rotunda*. It is present in pools at most exposed sites on the English coast as far east as Start Pt.; it is not now absent at Wembury ('Blackstone Pt.') as stated by Fischer-Piette (1936). On the French side it is quite abundant in the two outer Channel Islands, Alderney and Guernsey, and on the north coast of Cotentin, but does not occur east of Barfleur. It is often found as a distinct zone at the upper limit of *Himanthalia* in France and the Channel Islands, but we have not observed this form in England, although it was noted at a few stations by Fischer-Piette.

GENERAL TRENDS OF THE DISTRIBUTIONS

East-west trends

There is a clearly marked east-to-west trend in the distributions of many of the animals and plants which have been described. In nearly all such species not only is the general trend from east to west the same, but the range and even the detailed features show that a remarkable similarity exists between the British and French coasts. For example, *Balanus balanoides* becomes gradually less common west of the Cherbourg peninsula and of the Isle of Wight as *Chthamalus stellatus* increases, and markedly less common west of Start Point and Sillon de Talbert. The eastern limits on the two sides of the Channel of *Patella intermedia*, *P. aspera*, *Anemonia sulcata* and *Balanus perforatus* are almost identical. Perhaps the most striking feature of the general similarity between the two coasts is the sharp faunal and floral discontinuity existing on both sides at the junction of the eastern and western basins. On the British side the limits of *Chthamalus stellatus*, *Balanus perforatus*, *Littorina neritoides*, *Gibbula umbilicalis*, *Patella intermedia*, and *P. aspera* occur at or very close to the Isle of Wight, the limits of *Monodonta lineata* and *Himanthalia* somewhat farther to the west, while *Anemonia sulcata*, though very common in parts of the Isle of Wight, has been found at only one station farther east. The critical nature of these limits is indicated in Fig. 4. A similar critical change occurs at Alderney and on the Cotentin peninsula, where *Chthamalus stellatus*, *Balanus perforatus*, *Patella depressa*, *P. aspera*, *Paracentrotus lividus*, *Haliotis tuberculata*, *Laminaria ochroleuca*, *Saccorhiza polysichides*, *Alaria esculenta*, *Himanthalia elongata* and *Bifurcaria rotunda* all have their eastern limits, although some species, e.g. *Monodonta*, *Littorina neritoides* and *Anemonia* extend a little to the east (Fig. 5).

These sharp biological limits midway along the Channel coincide with the topographical limits separating the two basins. Thus the western basin has a rich fauna including many Atlantic and Lusitanian–Mediterranean forms together with Boreal and Celtic species, while the eastern basin has a depleted fauna from which most of the southern elements are missing.

North-south differences

Differences between distributions of animals on the north and south coasts of the Channel are less conspicuous than those between the east and west basins. The differences that exist are due to the presence of more southern forms on the French coast. *Gibbula umbilicalis* and *Littorina neritoides* have a greater range eastwards on the French side: other forms which are generally more abundant are *Bifurcaria tuberculata*, *Laminaria ochroleuca* and *Gibbula magus*. Several species are found exclusively or almost exclusively on the French side, such as *G. pennanti*, *Haliotis*, *Paracentrotus*, and also species not included in the present survey such as *Pollicipes cornucopiae*[1] and possibly *Pachygrapsus marmoratus*, which was at one time present in the Roscoff area. The only species which appears to be more abundant on the British coast is *Chthamalus stellatus*. It is interesting to note that this species can occur very high up in the intertidal zone, while those forms restricted to the French side are mainly found towards low-water mark.

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[1] There is a single record of *Pollicipes* from Land's End (specimens in British Museum).

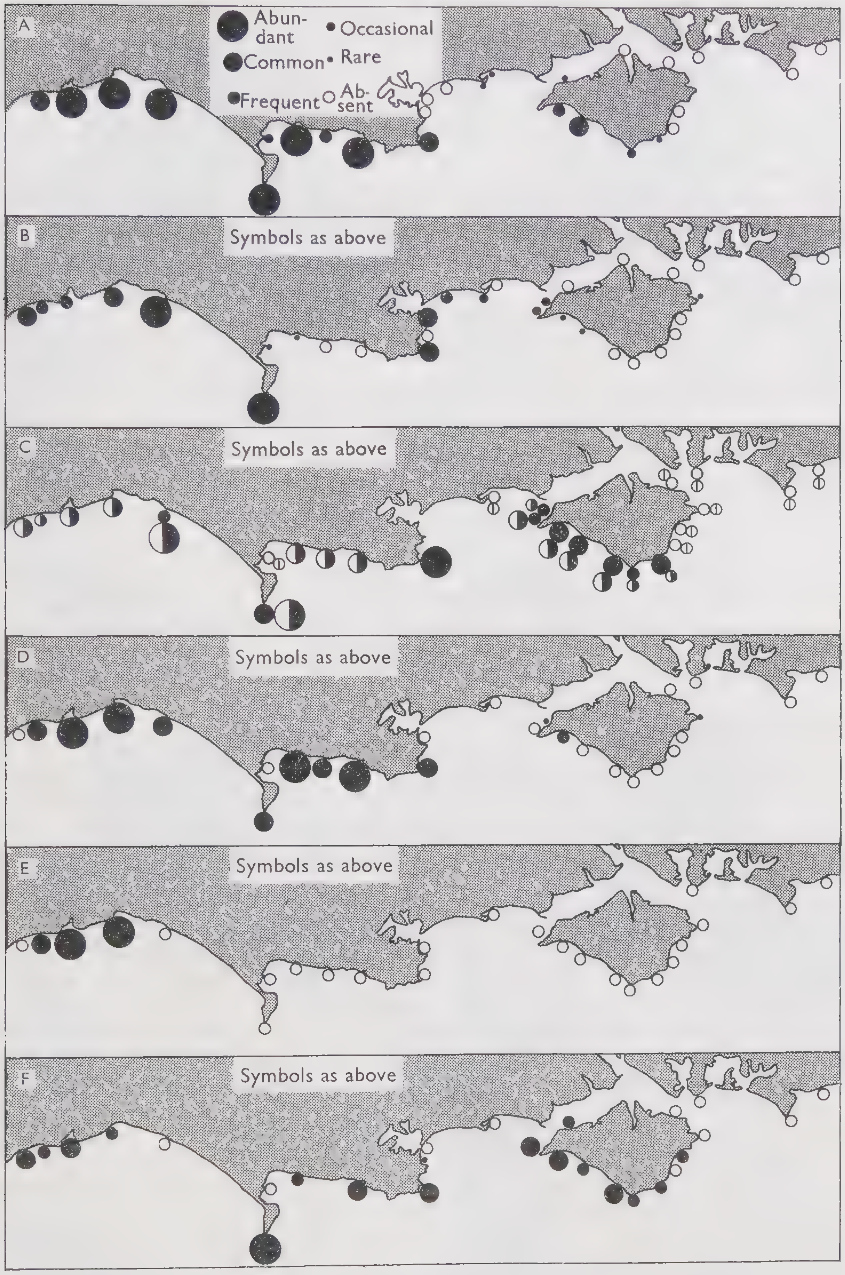
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Fig. 4. The critical region on the English coast where many of the southern forms have their eastern limits. A, *Chthamalus stellatus*; B, *Balanus perforatus*; C, *Patella aspera* and *P. depressa*, the latter shown by half-black or barred symbols; D, *Gibbula umbilicalis*; E, *Monodonta lineata*; F, *Littorina neritoides*.

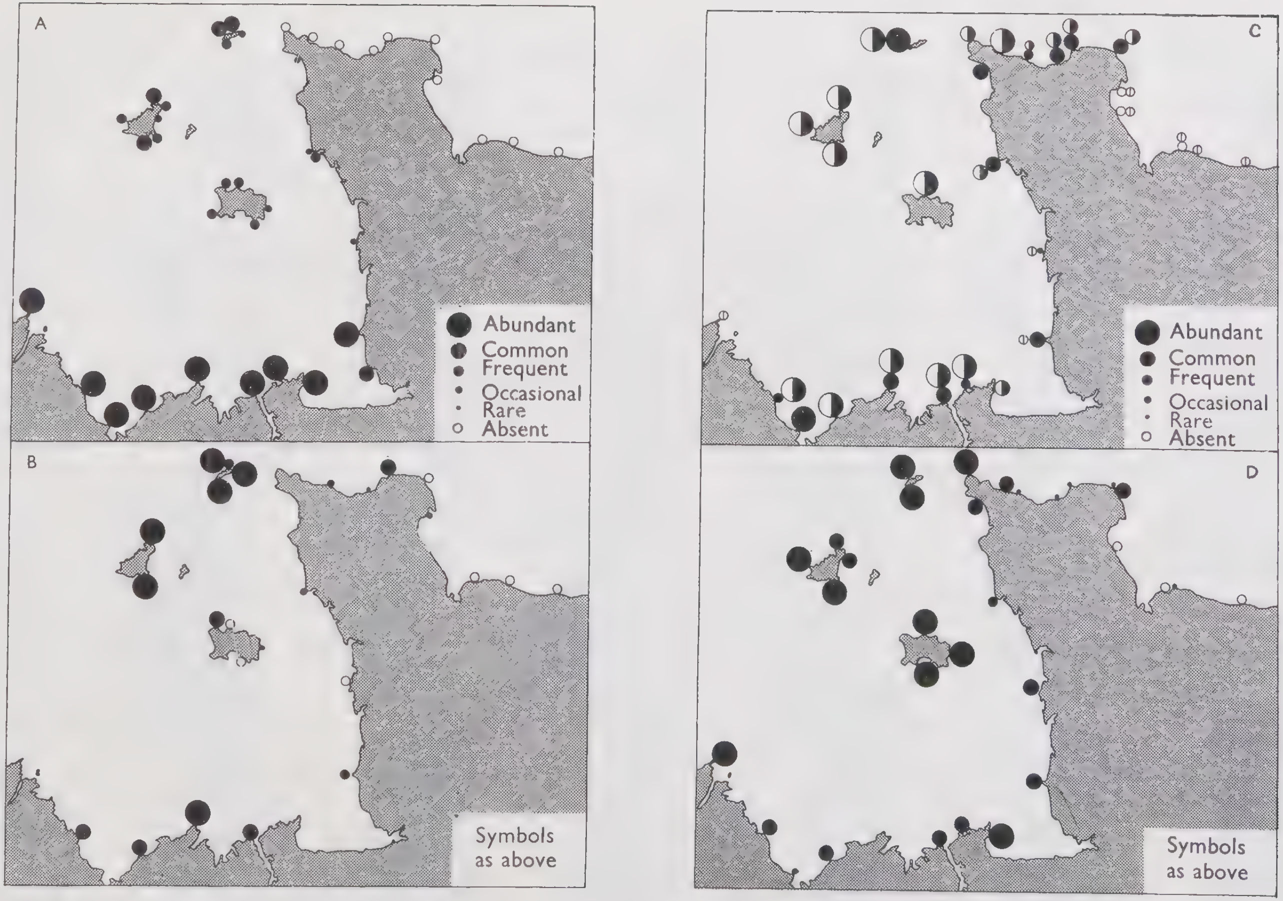
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Fig. 5. The critical region on the French coast, showing the limits of: A, *Chthamalus stellatus*; B, *Balanus perforatus*; C, *Patella aspera* and *P. depressa*; and D, *Monodonta lineata*. The half-black or barred symbols refer to *Patella depressa*.

RELATION BETWEEN DISTRIBUTIONS AND ENVIRONMENTAL FACTORS

We have already described the major environmental variables found in the Channel. In this section they are discussed as controlling influences on the distribution of the organisms.

The animals and plants which share the main trend in distribution from east to west are varied in many respects. First, their requirements differ in regard to exposure. Some, such as *Littorina neritoides*, *Chthamalus stellatus*, *Paracentrotus lividus*, *Patella aspera*, and *Alaria esculenta*, grow best in rocky wave-beaten situations, while others, such as top-shells *Monodonta lineata*, *Gibbula umbilicalis*, *G. pennanti* and *G. magus* occur predominantly in some degree of shelter. The greater expanse of rocky shore, and the heavier swell at the western end of the Channel cannot therefore be the only, nor indeed the main factors. At least one species not included in our survey, which requires the proximity of a sandy or gravelly environment, *Sabellaria alveolata*, is abundant in many areas at the western part of the Channel and is replaced by *S. spinulosa* to the east.

Secondly, they differ in their relation to tide level. Some of those confined to the western half of the Channel, such as *Anemonia sulcata*, *Gibbula magus*, *Paracentrotus lividus* and *Bifurcaria tuberculata*, are found mainly at the lower levels or in pools, while others such as *Chthamalus stellatus* and *Littorina neritoides*, are found high up the shore. It is not possible therefore to attribute the general trend to differences in the tidal regime such as the occurrence of equinoctial spring tides at midday in the western basin and at morning and evening in the eastern basin.

Thirdly they differ in their nutrition. The plants are, of course, autotrophic. Many of the animals, such as the limpets *Patella aspera* and *P. depressa*, the periwinkle *L. neritoides*, the top-shells *Monodonta lineata*, *Gibbula umbilicalis* and *G. pennanti*, and the urchin *Paracentrotus lividus*, are surface scrapers, though their nourishment must differ considerably with their tidal zonation and particular habitat. Others, such as the barnacles, can feed on suspended matter including a wide range of particle size (Southward, 1955*a*). *Anemonia sulcata* is a macrophagous carnivore, and *Hemioniscus balani* a parasite. The distributions cannot therefore be attributed to a greater abundance of food organisms in any particular habitat.

Lastly the organisms showing the east-west trend differ in their life histories. The great majority have planktonic stages, but there are without doubt great differences between them in the periods of their development in the sea, although the precise duration of many is not known. All the barnacles, *Paracentrotus*, and probably most of the littorinids have relatively long planktonic phases, while the limpets take only a few days to metamorphose (Lebour, 1937; Thorson, 1946; Dodd, 1957). The anemones are viviparous, while some of the algae have sporophyte generations which alternate with a small gametophyte. It follows that biological factors present in the sea which influence larval development cannot exercise an important influence on the distributions as a whole.

Effect of temperature

Of the few environmental factors which can be considered as having possible influences on such widely varying organisms, temperature, which may affect all stages of the life history, is probably the most important. Since the western end of the Channel is warmer on average and certainly less severely cold in winter, the species which are plentiful there should be those which are either unable to compete successfully with cold hardy forms or which suffer directly from exposure to the cold; that is forms with a southern or Lusitanian distribution. Conversely, since the eastern end of the Channel is much colder in winter, the forms predominating there must be tolerant of cold conditions. Table 1 (p. 159), which summarizes the known distributions and other relevant details of the species which have been studied, shows that the majority of the forms confined to the western basin of the Channel are in fact those of southern distribution. Of the species whose distribution extends well to the north of the British Isles, the majority are found in similar abundance in both basins, though Arctic forms such as *Balanus balanoides* are less common in the extreme west. The Arctic form *Alaria esculenta* is exceptional in occurring only in the western part of the Channel. It is possibly so dependent upon wave-beaten sites that it is excluded from the eastern basin, even though it has its southern limits just south of the Channel.

The differences between the north and south coasts of the Channel may also be attributed to temperature differences. The sea temperature is higher in winter on the French side of the western basin, and in summer is distinctly higher in the Gulf of St Malo and the Baie de Seine. Thus a number of animals and plants of southern origin penetrate for varying distances along this coast, although they are not represented, or are very sparse, on the coasts of South Devon and Cornwall. Others are able to extend farther eastwards on the French side than on the English side (see above).

There are certain objections, however, which might be raised against the view that the temperature gradient is the predominant factor influencing distributions in the English Channel.

The first difficulty arises from the generally accepted view, put forward by Apellöf (1912), Orton (1920), and Runnström (1929), that marine organisms are restricted primarily by the temperature prevailing during the breeding season, for most animals can live at considerably greater extremes of temperature than those which are tolerated during the reproductive period. Thus, for example, many animals found near their northern limits in south-west Britain breed only for a short period in the summer months, so that their fecundity is progressively reduced with fall in summer temperature. There is, moreover, little doubt that land-locked areas, having increased summer temperatures, offer especially favourable environments (Southward & Crisp, 1954*b*). Yet, as will be seen by comparing the summer and winter isotherms with the general trend of the distribution maps, the correlation between distribution and temperature applies not to the maximum reached in summer, which would be thought to be critical for reproduction, but rather to the winter minima. Winter conditions can have little relevance to the reproductive state or to the larval stages, for as shown in Table 1 all but three southern forms breed in summer. The difficulty cannot satisfactorily be resolved by assuming that winter temperatures in the eastern half of the Channel are lethal to the adults of these species, for there is no evidence of such mortality. Experimentally it has been demonstrated that some of the southern forms are killed at temperatures that are not uncommon on the land at the eastern end of the Channel. But, as the accompanying paper on this subject shows (Southward, 1958), the more extreme values of low air temperature recorded will be rarely experienced by the animals on the shore. Possibly the less extreme temperatures that are found on the shore might be shown to have debilitating effects in long-term experiments, but against this is the fact that one species at least (*Chthamalus stellatus*) can survive transplantation to the north and east of Britain, over-winter, and produce nauplii the following summer (Crisp, 1950). If mortality occurred as a result of cold, species which extend throughout the greater part of the intertidal range in south Devon, such as *Chthamalus stellatus* and *Littorina neritoides*, would be expected to be killed predominantly at their upper limits where the duration of frost exposure is greater. In fact, however, these two species are absent from the lower levels in cooler localities and at their extreme eastern limits are found only high up the shore.

These difficulties arise through seeking a temperature-vulnerable phase in the life history, probably an unwarranted simplification. All stages in the life history are influenced by temperature. Not only must the adults attain a breeding condition and the larvae develop successfully, but the post-larvae and young adults of most southern forms must also survive and grow sufficiently during the coldest part of the year. Therefore an equably warm environment such as occurs in the western half of the Channel may be better for southern forms than one of extremes, despite possibly higher temperatures at the breeding season. There may, in fact, be no direct influence of temperature at all. If the survival of the species depends upon its rate of growth or feeding in competition with other forms, temperature may operate decisively on this competition well above the level at which it would be directly lethal (cf. Southward, 1955*b*, 1957). We have described elsewhere how the generally warmer conditions in south-west Britain may favour a southern form *Chthamalus stellatus*, while towards the north and east it is displaced by *Balanus* *balanoides*, a northern form occupying a similar niche (Southward & Crisp, 1956). Barnes (1956) has evidently reached the same general conclusion. The evidence therefore suggests that the presence of some, perhaps the majority, of the southern forms in the western half of the Channel but not farther east is not simply because they can survive there during the milder winter, but rather because the temperature is favourable to them for a sufficiently long period during the year. That the recent climatic amelioration seems to have been accompanied in the Channel by an increase in range and abundance of at least three southern species tends to support this view. Further, the hypothesis that temperature influences animals and plants throughout the year, and during all stages in their life history, is necessary to explain certain minor trends in distribution as, for example, the variation in the abundance of *Chthamalus stellatus* and *Balanus perforatus* in the Gulf of St Malo (p. 171). In this area there is considerable variation from place to place in the range of temperature throughout the year, but it must on average be sufficiently high for southern forms. The abundance of these two southern species in the two outer islands, Alderney and Guernsey, can be attributed to the more equable temperatures there during the cooler part of the year; this probably assists them in competition with the northern species, *B. balanoides*, which is somewhat less abundant than it is in Jersey and other parts of the Gulf. However, the same two southern species are also abundant on the southern shores of the Gulf; their abundance there must be attributed to the beneficial effects of heating-up of the shore and shallow water during the summer, and its influence on breeding and larval output (cf. Southward & Crisp, 1954*b*). The beneficial effect must be so great that it enables the species to overcome the difficulties imposed by the very much colder conditions during the winter. Jersey lies between the two areas in temperature regime; its shores are cooler in summer than those of the mainland, and it is colder in winter than the outer islands. Presumably the summer temperature is not high enough to allow sufficient increase in breeding activity to compensate for conditions during the rest of the year.

Critical limits

There are other aspects of distribution that cannot be explained by the temperature hypothesis alone, notably the existence of sharp discontinuities affecting several species together. These discontinuities are found at the Isle of Wight and the north coast of Cotentin, at the region of separation of the eastern and western basins. Examples of similar critical limits are found in Anglesey (Crisp & Knight-Jones, 1955).

The critical limits in the Channel do not coincide with closely approximating isotherms (Figs. 1, 4, 5). If temperature alone were controlling distribution of the southern species the limits of different species would be expected to lie at a series of geographically separated regions, the position of each limit being related to the temperature tolerance of the species in its natural habitat. It is very unlikely that the tolerance limits would then be nearly identical for so many of the species studied. Therefore these sharp faunistic limits point to some factor additional to temperature acting as a barrier.

Effect of substratum

The shores of the eastern basin of the Channel differ from those farther west (p. 164), the infrequent outcrops of harder rock and the prevalence of beaches of scouring material making conditions less suitable for rock-living organisms. These conditions influence adversely all such organisms, but any which are near extinction through other factors, such as temperature, may be unable to extend beyond the point where suitable rock becomes scarce. That this is so is strongly suggested by the fact that the fauna of the eastern section of the Channel is an impoverished one. All the species represented there, so far as we know, are found in the western basin, though some in less abundance. Many species found in the west are, however, absent from the eastern part of the Channel. These species are mainly, but not all, of southern origin. Similarly, many species of algae, for example *Bifurcaria*, *Himanthalia*, *Saccorhiza* and *Alaria*, the latter a northern form, are absent. This poverty of algae must surely be related in part to lack of sufficient rock substratum, to opacity of the water, and to the damage due to scour. The relative lack of rocky substrata and the extent of the scouring that may occur in the eastern basin probably contributes to the absence of the more southern barnacles, and to the scarcity or absence of forms such as *Patella aspera* and *Littorina neritoides*, which can live under the cold conditions of the Yorkshire coast. Thus, it may be that to forms approaching their limit of tolerance of other conditions, including temperature, a change in the nature of the shore may present a simultaneous physical barrier.

However, although the critical faunistic limits correspond roughly with the beginning of extensive areas of sand and shingle, certain of the species terminate to the west of the point where the shore character changes. For example, *Chthamalus* is not found on the north coast of Cotentin, where ample rock is available, while apparently suitable beaches of large boulders occur in Dorset and Normandy eastwards of the limits of *Monodonta*. In the Isle of Wight several of the species studied do not penetrate beyond the southern tip, though apparently suitable reefs exist at the eastern end of the island. Populations growing near the eastern boundary of a rocky shore can clearly be recruited only from the west, but this should not in theory reduce the population density below about half that of regions where recruitment can occur from both directions, assuming even dispersal of larvae.

There are two other factors which may account for the position of these critical faunistic limits more precisely: aspect and dispersal.

Aspect

The need to shelter half-hardy garden plants from north and east winds is well known. The same is likely to hold for the sessile or nearly sessile plants and animals of the intertidal zone. Indeed the effect is probably more pronounced on the shore than it is inland because the cooling effect of strong cold winds from east or north will be enhanced by evaporation as the sea ebbs and the animals are exposed to the air. On a south- or west-facing shore the strongest winds will be associated with mild weather, but on a north-, and especially on an east-facing, shore the strongest onshore winds will be from a cold quarter, so that the greatest cooling by evaporation will occur simultaneously with low air temperature. North-facing shores will also receive less warmth on account of the lower angle of incidence of the sun; they may be almost without sunlight in winter if the slope is steep. The distribution maps show that many of the limits of southern forms coincide with a change in aspect of the coast. *Chthamalus stellatus*, *Balanus perforatus*, *Gibbula umbilicalis*, *Littorina neritoides* and *Patella intermedia* all disappear near St Catherine's Pt., Isle of Wight, where the aspect of the coast changes from south-west to south-east; on the Cherbourg peninsula *Chthamalus* disappears near Cap la Hague, where the aspect changes from west to north, while *Patella depressa*, *P. aspera*, *Himanthalia*, *Bifurcaria* and *Saccorhiza*, and to a large extent *Balanus perforatus* disappear around Barfleur, where the aspect changes from north to east. A notable example of the apparent influence of aspect on a species of northern character is found in west Cornwall (Fig. 6). Here *B. balanoides* is virtually absent from the south coast, rare on the west-facing portion, and abundant on the north-facing region of the coast. Although not shown on the map, the species becomes less common farther east on the north Cornish coast, where it gains a more westerly aspect.

Effects of currents

Another factor which may cause several species to undergo a sudden change in abundance is the influence of currents on dispersal of the planktonic larvae. Intertidal animals with free-swimming larvae must suffer loss of recruitment whenever the larvae are carried too far out to sea and fail to return to the coast. The amount of loss will depend on how far the tidal or residual currents carry water masses offshore, and will increase with the strength of these currents. When the loss of recruitment becomes critical the species must disappear, for there are usually minimal densities of adults below which the population cannot maintain itself without outside assistance (Crisp, 1950; Crisp & Southward, 1953; Crisp, 1958: see also Darwin, 1872; Andrewartha & Birch, 1954).

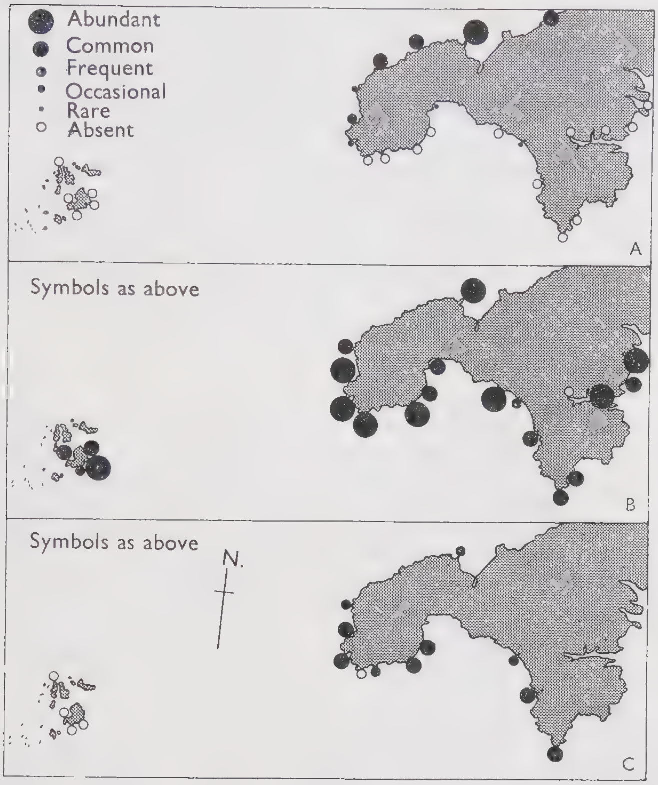

Fig. 6. Part of west Cornwall and the Isles of Scilly, showing the distribution of: A, *Balanus balanoides*; B, *B. perforatus*; and C, *Patella depressa*.

Dispersal by tidal streams

In the Channel, the main tidal streams are greatest between the two basins (see p. 163), while offshore races with turbulence occur at headlands. Therefore the greatest loss of larvae by dispersal would be anticipated at headlands such as St Catherine's Pt., Portland Bill, and off the points of the Cherbourg peninsula and Alderney. Not only must dispersal be increased in this region, but also the diminished tidal range, especially on the English side, may restrict the available stocks of adults, since these are necessarily confined to a narrower zone on the shore.

Change in aspect and increased dispersal are probably both important in determining faunistic boundaries. The fact that both on the south-facing British shore and on the north-facing French shore many of the critical faunistic limits coincide with headlands lying midway between the two main basins suggests that larval dispersal may be a more important factor than aspect.

The influence of dispersal should be equally harmful to species spreading from either direction. It is interesting therefore to note that Peveril Point, Portland Bill, and Cap la Hague present barriers to the westward spread of the immigrant barnacle *Elminius modestus* (Crisp, 1958).

Only species with long planktonic larval stages will be seriously reduced by dispersal. It may therefore be significant that the top-shells *Gibbula umbilicalis* and *Monodonta lineata*, which probably have short larval stages, continue eastwards along the French coast, though in diminished numbers, past the Cherbourg peninsula. The former extends as far as the Straits of Dover. *Anemonia sulcata*, which can reproduce by fission, extends a short distance into the eastern basin on both sides of the Channel.

Dispersal by residual drift

Another effect which should be considered in connexion with larval dispersal is the influence of residual drifts. The general easterly movement of water will carry larvae up-Channel. To a greater or lesser extent, depending on the form of the coastline, the young are thus recruited from stocks to the west rather than from those to the east. Similarly, larvae may be carried round the Brittany peninsula from south to north. During the life of most larvae (anything from 2 to 30 days) the residual drift near the shore is likely to be small and to be masked by tidal streams, counter eddies, and retention in pockets. However, at extreme westerly peninsulas or small islands where westerly recruitment is not possible one might expect considerable impoverishment of those elements of the fauna which have a long planktonic phase. This effect certainly obtains for extremely isolated islets such as Rockall (Crisp, 1956). It is surprising that so little impoverishment occurs at such places as north-west Brittany, Cape Cornwall, Lizard Head, Eddystone Rocks, etc. The presence, for example, of good barnacle settlements in these areas suggests that there are coastal drifts running counter to the main eastward drift, and able to carry larvae round these headlands. Certain species, however, become sparse at the western extremity both of Brittany and Cornwall, and more particularly in Ushant and the Scilly Isles, notably *Balanus balanoides* (absent from Scilly), *B. perforatus* (not at all abundant on Ushant, Scilly, or the Lizard), *Patella depressa* (absent from Scilly, rare on Ushant, not abundant in Finistère or west Cornwall), *Littorina littorea* (rare in Scilly and Ushant), and *Mytilus* (not studied in this survey, but known to be uncommon in Scilly and Ushant). *Balanus balanoides* and *Littorina littorea* are relatively northern in distribution and may be affected by the warmer maritime conditions in these areas, but this is not likely for the other species.

One of us (A. J. S.) has recently obtained further evidence relating to larval dispersal off west Cornwall and the Isles of Scilly, where a series of plankton samples were taken over a limited period in March 1956 (Fig. 7). The samples were taken for the specific purpose of determining whether the larvae of

Balanus balanoides were present off Scilly during the short breeding period of the species. However, the distributions of the larvae of other species of barnacles found in the samples proved to be pertinent to the present work and are also illustrated. The boundaries of both *Verruca stroemia* and *Balanus crenatus* were very sharp, few larvae being found at distances from the coast exceeding a few miles, even though easterly winds of force 3–6 prevailed during the survey. The western limits of the cirripede larvae coincided with the main boundary between relatively oceanic plankton organisms (*Sagitta elegans*, *Candacia*) and neritic forms (*Podon*, *Evadne*, *Sagitta setosa*). The adults

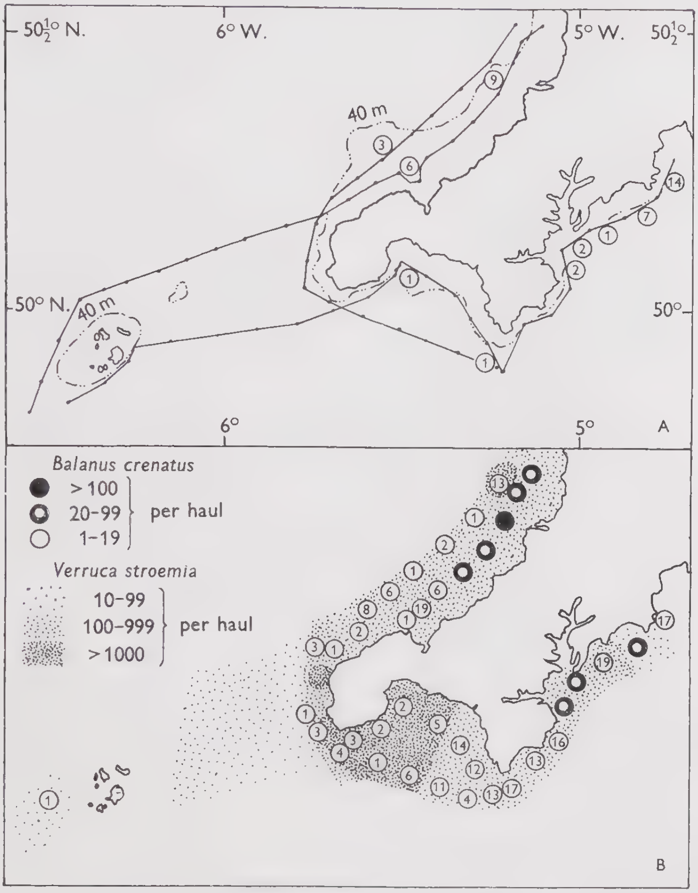

Fig. 7. Distribution of cirripede nauplii off west Cornwall and the Isles of Scilly as shown by samples taken with a standard Hardy plankton indicator, 27–29 March, 1956, towed at approx. 20 m deep at 8·5 knots. A, numbers of *Balanus balanoides* per haul of 4 nautical miles; B, numbers of *B. crenatus* and contours for *Verruca stroemia*. Positions of hauls, and depth contours are shown on A.

of both *Balanus crenatus* and *Verruca* are found in the infralittoral zone, but the larval distribution indicates that while *Verruca* may extend some way offshore in this region, *Balanus crenatus* is almost entirely confined to the coastal belt. The maps show that larval populations of all three species form only a narrow belt at extreme western points, but accumulate over a broader area and in greater numbers in Mounts Bay. The density of most of the larval populations also increases in an easterly direction from Land's End. Certain aspects of this distribution, for example the greater abundance of *B. balanoides* larvae at the more easterly stations and in the vicinity of St Ives, follow directly from the distribution of the adults. The general pattern is entirely consistent with the view that larvae are dispersed by an eddy diffusion modified by a general north-easterly drift.

In considering the influence of dispersal on the absence or scarcity of intertidal or neritic animals, the duration of the free-swimming stage and the period during which breeding occurs are of primary importance. If the free-swimming life is long, a species will be more widely dispersed and more readily pass across a barrier of deep water, unless the currents are always set against it; on the other hand, it would be more likely to disappear through excessive dispersal from a small island effectively isolated from the mainland. The shorter the period over which larvae are liberated, the less likely will the animal recolonize new territory or maintain its hold in an isolated place. The short breeding season of *Patella depressa* and *Balanus balanoides* may account in part for their complete absence from Scilly. However *Patella depressa* has probably only a short free-swimming period, and may not therefore have ever been able to reach the islands, just as it is believed to have been similarly prevented from crossing the Irish Sea (Crisp & Southward, 1953). *Balanus balanoides* has a long larval life; its absence is more likely to be due to the small density of adults on the adjacent coasts, the usual set of the residual current carrying the larvae eastwards. It would be in danger of elimination by excessive dispersal, even if as a result of exceptional west-going drifts it gained a foothold.

An effect that may be associated with residual drift is the tendency of water masses to move clockwise in the northern hemisphere under Corioli's force. This might result in a greater eastward movement, and better retention of larvae, near the French coast. Southern forms should therefore be carried farther westward on this side of the Channel, though the magnitude of the effect is difficult to determine.

Faunal continuity

Intertidal forms can spread much more readily along a continuous shoreline than across sea passages (Crisp & Southward, 1953; Southward & Crisp, 1954*b*). Hence the continuity of the French coast of the Channel with the Atlantic coasts of France and Spain may allow the survival in the Channel of some species which, in isolation, would disappear. The case of *Pachygrapsus marmoratus*, which was able to establish itself locally only during favourable periods (Prenant, 1929), illustrates the importance of continuity. It is possible that species such as *Gibbula pennanti* and *Paracentrotus lividus*, which are absent from the English side, can be replenished more easily on the French side by larvae from outside the Channel after periods of unfavourable temperature. Purely infralittoral forms, such as *Octopus vulgaris* (Rees & Lumby, 1954), may also be influenced strongly by continuity.

Effect of suspended particles in the water

We have noted briefly that some of the algae may be excluded from the eastern part of the Channel partly by the opacity of the water. To these organisms such a factor may be more important than aspect or dispersal, and needs examining in more detail. The reduced amount of light received in the lower intertidal zone, where the water is silty, is probably most detrimental to the infralittoral and lower midlittoral species. The fucoids can withstand greater exposure to air, and hence can occur at higher levels on the shore, where they remain sufficiently illuminated for photosynthesis (cf. Burrows & Lodge, 1951). Forms such as *Himanthalia* and *Bifurcaria* cannot occur at higher levels out of the water, and will thus be unable to compensate for the reduced illumination of silty water. They will grow best where the water is clear and the tidal range smallest, for under these conditions they can remain permanently immersed with minimum loss of illumination (cf. Fischer-Piette, 1936). Any effects of reduced illumination caused by particulate matter in the eastern basin will be slightly accentuated by differences in the tidal regime. In the western basin low water of spring tides, and high water of neap tides occur at midday, while the opposite is true for the eastern half of the Channel. This difference means that, on average, there is a slightly thicker layer of water over the intertidal zone during daylight hours in the eastern basin in places where the tidal range is identical.

It is interesting to note that the absence or scarcity of *Himanthalia* and *Bifurcaria* in the eastern part of the Channel is paralleled in the inner parts of Lyme Bay and the Gulf of St Malo (Fischer-Piette, 1936; Lami, 1941). The shores of these two regions are erodible or scoured, there is more silt in suspension, and the tidal range is greater than farther east (in Dorset, north Cotentin and the Channel Islands) where these algae reappear in some abundance.

Effects of other factors

Other factors have so far not been adequately demonstrated, and at present there appears no need to invoke them to account for differences in distribution (see p. 158), though their possible existence has been frequently postulated. Smith (1953) has suggested that adsorption of trace constituents on particulate matter may contribute to the poverty of the fauna of the eastern part of the Channel and southern North Sea. Because of the limited adsorption capacity of such particles it is difficult to see how this process could continue unless the adsorption was a prelude to destruction or transformation of the substances adsorbed. This is by no means impossible, for particulate matter is the normal site of bacterial action.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

It has been shown that the general distribution of many of the organisms investigated may be explained by differences in temperature along the Channel. The existence of critical limits for several species rather than a series of graded boundaries for each species can be accounted for by the modifying effects of additional environmental factors, especially the character of the substratum, the effect of water movement on the dispersal of larvae and the aspect of the shoreline.

It seems unlikely that further faunistic work can establish with certainty our views as to the causes of the distribution. There are too many independent and unmeasurable variables. However, careful and regular observations in certain areas, and further complete surveys from time to time may make it possible to relate the abundance of certain animals to climatic trends, and hence to temperature, or with some other variable.

Further work on the distribution of animals in other habitats would be useful, especially habitats such as sands, muds, crevices, which offer similar conditions and escape certain of the possible modifying influences such as wave-exposure, scour, and aspect. In particular, investigations could well be made of the bottom fauna of representative stations along the length of the Channel. It is clear from the work of Rees (1950) and Rees & Lumby (1954) that the distribution of *Octopus vulgaris*, a southern form, shows east-west and north-south trends similar to those of many intertidal organisms. This species, moreover, shows fluctuations in abundance of adults and larvae correlated with fluctuations of sea temperature, especially of the winter months (Rees & Lumby, 1954).

East-west distribution trends are also found among planktonic animals in the Channel. There is, for example, a sharp boundary in the western basin between relatively oceanic species to the west and neritic species to the east (Russell, 1935). This line, however, lies well to the west of the boundary of the intertidal organisms, with which it appears to have little relation. A somewhat nearer approach to the intertidal boundaries is shown by the line dividing the copepod *Centropages typicus* in the western basin from the related species *C. hamatus* which is largely confined to the eastern basin and the Gulf of St Malo (Cushing, 1957). The different distribution of these two species has been attributed by Cushing to differences in water currents and bottom deposits in the two basins, factors we have already suggested may be responsible in part for some of the distributions of intertidal organisms. More correlation between temperature and east-west trends may be found with the pilchard, *Sardina pilchardus*, which is largely confined to the western basin (and Celtic sea) in winter, migration into, and spawning in the eastern basin occurring only during the warmer summer months (see Cushing, 1957).

Only further work on the fluctuations of the boundaries of the planktonic organisms can show whether there is, in fact, any relation between the distributions of these species and of intertidal and bottom-living organisms.

It seems unlikely that experiments on the tolerance of animals to temperature extremes will be immediately relevant to understanding the effect of temperature in distribution. This is more likely to be furthered by studies of the behaviour, nutrition and reproduction of the various animals and plants with special regard to their efficiency and success in natural competition. The latter calls for investigations of suitable animals kept for prolonged periods at a series of temperatures and with various conditions of feeding.

The effects of dispersal on the free-swimming stages is a problem that has been given too little attention in the past and one which offers results equally valuable to the biologist and the hydrographer. Each larva is a clock, set in motion at the moment of liberation from the adult and carried passively from its place of origin by tides and currents. Taken in conjunction with the distribution of adults, quantitative measurements of planktonic larvae can give information on the dynamics of distribution and dispersal of the animals as well as on the direction of local water movements.

During the course of the work we have had the assistance, from time to time, of many friends and colleagues to whom thanks are due: in particular we should like to mention Dr Eve C. Southward, Mr L. W. G. Jones and Mr E. Norris. We are indebted to the Browne Fund of the Royal Society for grants which assisted us in carrying out parts of the field work. Additional financial aid was received by one of us (A. J. S.) from the Department of Scientific and Industrial Research (Senior Research Award), the University of Liverpool and the Marine Biological Association.

SUMMARY

The English Channel may conveniently be divided into a western basin and an eastern basin separated by a line drawn approximately between the Cherbourg peninsula and the Isle of Wight. In these basins, the environmental factors are very different. Thus the water of the western basin is on average warmer and slightly more saline than that of the eastern basin and its temperature variation is less extreme. The French side of the Channel is warmer than the English coast only in the western basin. The tidal oscillations in the two basins

are out of phase with each other and the strongest tidal currents occur where the two basins join. The main residual flow is up-Channel with minor coastal eddies.

The shores at the western end of the Channel are generally rocky, they are close to deep water, and so are exposed to frequent heavy swells. The shores of the eastern basin are generally erodible, and accumulations of sand and shingle tend to reduce the force of the waves. The water of the eastern basin generally contains more suspended matter and the shores are usually scoured by sand and shingle.

The distribution of the following animals is given in some detail and compared with earlier records: *Anemonia sulcata*, *Actinia equina*, *Chthamalus stellatus*, *Balanus balanoides*, *B. perforatus*, *B. crenatus*, *B. improvisus*, *Elminius modestus*, *Verruca stroemia*, *Hemioniscus balani*, *Patella vulgata*, *P. depressa*, *P. aspera*, *Monodonta lineata*, *Gibbula umbilicalis*, *G. pennanti*, *G. cineraria*, *Littorina saxatilis*, *L. littoralis*, *L. littorea*, *L. neritoides*, *Paracentrotus lividus*. The distribution of certain other species is also discussed: *Balanus balanus*, *Haliotis tuberculata*, *Gibbula magus* and *Calliostoma zizyphinum*. The distributions of the following plants are briefly discussed: *Laminaria ochroleuca*, *L. digitata*, *L. hyperborea*, *L. saccharina*, *Saccorhiza polysichides*, *Alaria esculenta*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus spiralis*, *F. vesiculosus*, *F. serratus*, *Himanthalia elongata* and *Bifurcaria rotunda*.

The main trend in distribution is from east to west. Northern forms are mostly found throughout the Channel though commoner at the eastern end. Many southern forms common in the western basin fail to penetrate the eastern basin, and the fauna at the eastern end of the Channel is therefore impoverished as compared with the richer fauna of the western basin. Certain southern forms are found in the western basin only on the French side of the Channel; others extend farther eastwards on the French side than on the English side.

The trends in distribution are considered to be due to temperature differences. The eastern limits of southern forms are considered to result, not from a lethal effect of a minimum temperature, but from the generally lower efficiency of these animals in comparison with northern forms as the temperature falls. The substratum of most of the eastern basin of the Channel, especially on the English side, is inimical to many rock-living organisms, and it seems probable that this reinforces any adverse effects due to the lowering of average temperature.

The limits of many predominantly southern species occur somewhat abruptly on both north and south coasts of the Channel in the region lying roughly midway between the two basins. This concentration of faunistic limits is believed to be due partly to the changes in the aspect of the coast, which expose it to the north and east winds, and partly to excessive dispersal of larvae by tidal currents which are maximal in these regions.

The influence of residual drift may be important to the distribution of certain organisms at the extreme west of the Channel, viz. *Balanus perforatus*, *B. balanoides*, *B. crenatus*, *Patella depressa*, *Littorina littorea* and *Mytilus edulis*. *Balanus balanoides* and *Patella depressa* are absent from the Isles of Scilly.

The clarity of the water, the tidal range, and the tidal regime (i.e. time of high and low spring tides) appear important to certain algae such as *Himanthalia* and *Bifurcaria*. Heavy swell appears essential to *Alaria esculenta*, and this rather than temperature confines it to the western part of the Channel.

Further work on the behaviour, nutrition and breeding of certain of the animals from rocky shores at a series of temperatures seems desirable, together with a study of distributions of animals from other habitats. The mechanism of dispersal and the distribution of larvae of shore animals also deserve more attention.

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APPENDIX

TABLE 7. THE DETAILED DISTRIBUTION OF THE COMMONER INTERTIDAL ANIMALS IN THE CHANNEL

The table summarizes observations made over a number of years. During the course of the work our methods improved and the number of species under investigation was increased. As it was not possible to revisit all stations some areas will be found to have less detailed records than others.

The place names are taken from the largest scale map of the region available at the time; where only 1:250,000 maps were used, the nearest place name was taken. The survey of the English coast was spread over 1949–57: where it was possible to choose between counts or observations at a station, those made nearest to the date of the French parts of the survey have been tabulated. Dates of observations not used to complete numerical details of abundance are shown in brackets.

The type of shore at each station has been tabulated from the point of view of the relative hardness and extent of the rocky substratum, the angle of slope and the degree of shelter provided by offshore reefs. The letters used have the following meanings: R, rocks other than chalk; C, chalk rocks; B, boulders (over 10 cm diameter); G, gravel (10 cm to 2 mm diameter); S, sand (2 mm to 0·02 mm diameter); M, silt and clay (under 0·02 mm); P, piers, breakwaters, wharves and other artificial substrata. To the letters R and C are suffixed other letters thus: S, steep slopes (more than 25° from horizontal); F, flat rocks (e.g. Limestone and Sandstone flags); I, broken surfaces, all angles; E, reefs extending well out from the shore. The meanings of the symbols used to denote the abundance of the animals will be found in Table 2.

No.	Station	Description of shore	Dates of survey	*Anemonia sulcata*	*Actinia equina*	*Chthamalus stellatus*			*Balanus balanoides*			*Balanus crenatus*	*B. improvisus*	*B. perforatus*	*Elminius modestus*	*Verruca stroemia*	*Hemioniscus balani*	*Patella aspera*	*P. depressa*	*P. vulgata*	*Gibbula cineraria*	*G. pennanti*	*G. umbilicalis*	*Monodonta lineata*	*Calliostoma zizyphinum*	*Littorina littorea*	*L. neritoides*	*L. obtusata*	*L. saxatilis*	*Paracentrotus lividus*
						H.W.	M.T.	L.W.	H.W.	M.T.	L.W.																			
	Isles of Scilly																													
1.	Shipman Hd., Bryher	R, S	May 55	.	A	3·6	3·0	2·2	N	N	N	.	.	.	.	.	.	90 %	N	C	.	.	.	.	.	.	A	.	.	N
2.	Hugh Town, St Mary's	P, B, S	May 55	A	.	0·4	.	.	N	N	.	.	.	C	N	.	.	.	N	A	.	.	.	.	.	.	N	.	C	N
3.	Peninnis Hd., St Mary's	RI, B	May 55	.	.	A	A	.	N	N	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	A	.	.	N
4.	Porth Minick and Porth Hellick	B, G, S	May 55	.	.	2·6	2·0	1·6	N	N	N	.	.	F	N	C	.	.	N	.	.	.	A	A	.	N	A	C	C	N
5.	Darrity's Hole	R, S	May 55	.	.	1·8	4·8	1·7	N	N	N	.	.	A	N	.	.	.	.	.	.	.	.	.	.	.	A	.	.	N
6.	Blockhouse Pt.	RI, B	May 55	.	.	4·8	4·0	1·2	N	N	N	.	.	C	N	.	.	.	.	.	.	.	.	.		.	.	.	C	N
	Cornwall																													
7.	Cape Cornwall	RI, E	May 55, April 56	C	.	4·0	3·2	1·8	N	0·004	0·002	.	.	C	N	.	.	A	10 %	A	.	N	A	A	.	.	A	.	.	N
8.	Sennen Cove	B, S, P	May 55, April 56	C	C	2·8	3·2	2·3	N	0·01	0·03	.	.	A	N	.	0 %	A	20 %	A	.	N	A	C	.	.	C	.	.	N
9.	Nanjizal	RI, B	May 55	.	.	2·4	4·0	2·4	N	0·0002		.	.	A	N	.	.	A	22 %	A	.	N	C	C	.	.	A	.	.	N
10.	Porthgwrra	RI, B	May 55	.	.	3·2	2·4	1·4	N	N	N	.	.	A	N	.	.	.	N	.	.	N	C	.	.	.	.	.	.	N
11.	Logan Rock, Treen	RI, B, S	May 55	.	.	4·0	1·8	0·8	N	N	N	.	.	A	N	.	.	.	10 %	.	.	N	O	O	.	.	A	.	.	N
12.	Lamorna Cove	R, S	May 55	.	.	3·4	5·6	2·4	N	N	N	.	.	A	N	.	.	.	20 %	.	.	N	.	.	.	.	A	.	.	N
13.	Mousehole	B, S, P	May 55	R	.	4·8	2·4	0·6	N	N	N	.	.	C	N	.	.	.	20 %	.	.	N	A	A	.	.	A	.	.	N
14.	Penzance Harbour	M, P	May 50	.	.	A	.	.	N	R	.	.	.	C	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14.	Penzance Harbour	M, P	April 56, April 57	.	.	A	.	.	.	R	R	.	.	A	0·01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15.	Rinsey Head	RI, B	April 56, April 57	.	.	A	.	.	N	.	.	.	.	A	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16.	Porthleven Harbour	B, G, P	April 55, April 56	.	.	A	.	.	N	R	R	A	.	F	N	C	.	.	F	.	.	.	.	C	.	.	.	.	.	.
17.	Poldhu Cove	RI, E, B, S	May 51, April 55	.	A	4·9	5·4	3·1	N	N	N	.	.	C	N	.	.	.	40 %	.	.	N	A	A	.	.	A	.	.	N
18.	Porth Mellin	P	April 55	.	.	A	.	.	N	N	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
19.	Lizard Pt.	RI, E	May 51	.	.	A	.	.	N	.	.	.	.	N	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
19.	Lizard Pt.	RI, E	April 55, April 56	F	.	4·8	4·5	4·8	N	N	N	.	.	C	N	.	.	A	27 %	A	.	.	A	A	.	.	A	.	.	N
20.	Cadgwith	RI	April 55	.	.	A	.	.	.	N	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
21.	Gweek	M, P	May 50, April 55	.	.	N	N	N	N	N	N	N	N	N	4·0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
22.	Porth Navas	M, P	May 50, April 55, April 57	.	.	0·6	.	.	N	.	.	F	.	A	0·5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
23	Helford Passage	RI, G	May 50, April 55, April 57	.	.	A	.	.	N	N	.	.	.	A	R	.	.	.	.	.	.	.	A	A	.	.	.	.	.	.
24.	Durgan	RI, G	May 50, April 56	.	.	A	.	.	N	N	.	.	.	A	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
25.	Maenporth	RI, S	May 50, April 56	.	.	A	.	.	N	N	.	.	.	C	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

27. Flushing	G, M, P	April 56	.	.	A	A	A	N	N	N	.	.	A	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
28. Mevagissey	RI, P	April 56	.	.	A	A	.	N	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
29. Charlestown	P	April 56	.	.	4·0	3·2	.	N	0·06	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
30. Par	P	Dec. 50	.	.	A	A	A	N	N	O	.	.	C	O	.	94%	.	.	.	.	.	.	.	.	.	.	.	.	.
31. Polkerris	RI, B	April 55	.	A	4·0	3·2	2·3	N	N	R	.	.	A	0·01	.	72%	A	40%	A	.	N	A	C	.	.	A	.	.	N
32. Fowey	P, G	Dec. 50, April 56	.	.	3·2	2·9	4·8	N	R	N	O	.	A	0·16	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
33. Llansallos	RS	May 54	A	A	A	A	.	N	N	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	A	.	.	N
34. Nailzee Pt., Looe	RI, G, S, E	(Sept. 49), April 56	.	A	4·8	3·0	1·0	N	R	O–R	.	.	.	N	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
35. Looe Harbour	R, B, P	(Sept. 49), April 56	.	.	A	A	N	R	O	N	O	.	C	0·2	.	50%	.	.	.	.	.	.	.	.	.	.	.	.	.
36. Whitsand Bay	RS, S	Oct. 55	.	A	5·0	3·6	2·4	0·2	2·1	1·2	.	.	A	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	N
37. Amory Bight	RS	Dec. 54	.	.	7·5	4·4	1·6	0·01	0·16	0·05	.	.	A	0·08	.	.	.	.	.	.	.	.	.	.	.	A	.	.	N
38. Hen Pt.	B, M	Dec. 54	.	.	R	N	N	N	R	R	C	F	N	2·0	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S. Devon																													
39. Weirquay	B, M, P	Jan. 55	.	.	N	N	N	N	N	N	F	C	N	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
40. Tinside, Plymouth	RS, B	(Aug. 50), Dec. 54	.	.	3·4	2·4	0·3	N	R	N	.	.	C	C–F	.	10–60%	.	.	.	.	.	.	.	.	.	C	.	.	N
41. Laira, R. Plym	B, M, P	Mar. 51, Jan. 56	.	A	C	F	O	O–R	O–R	F–R	C	.	C	C–A	.	28%	.	.	.	.	.	.	.	.	.	.	.	.	.
42. Wembury	RSI, E	Feb. 55	A	A	5·2	2·8	0·4	0·2	1·1	0·3	.	.	A	R	F	20%	C	C	A	A	N	A	A	+	C	A	C	A	N
43. Hope Cove	RS, B, S, E	April 55	N	.	5·8	2·8	1·6	N	0·2	0·3	.	.	C	N	.	7%	.	50%	.	.	.	A	C	.	.	C	.	.	N
44. Salcombe, South sands	RS, S	Feb. 51, April 55, April 57	C	A	3·7	2·2	0·3	0·05	1·1	1·9	C	O	A	O	O	8%	F	17%	A	C	.	C	C	+	F	C	C	C	N
45. Salcombe, Charlton bridge	P, G, M	April 57	.	.	.	.	.	.	0·02	.	R	.	O	A	.	.	.	.	.	.	.	N	N	.	C	.	.	.	.
46. East Prawle	RI, E	April 55, April 56	.	.	4·8	3·2	1·3	0·04	0·6	0·4	.	.	A	N	.	.	.	15%	.	.	.	A	A	.	.	.	.	.	N
47. Start Pt.	RSI	April 57	.	C	2·5	2·7	2·0	N	0·03	0·1	.	.	F	.	.	0%	C	62%	A	.	.	.	.	.	.	A	.	F	N
48. Torcross	RF, G	April 50, April 57	.	C	C	.	.	.	O	.	.	.	O	N	.	.	C	15%	.	.	.	C	.	.	.	.	.	.	.
49. Blackpool	R, S, G	April 50, April 57	.	.	A	.	.	.	O	.	.	.	C	N	.	.	.	37%	.	.	.	.	.	.	.	A	.	F	.
50. Dartmouth	P, G, M	April 50, April 57	.	.	C	O	N	C	A	A	.	.	.	O	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.
51. Greenway	B, P, M	Feb. 51, April 57	.	.	F	R	N	C	A	C	C	O	O	C	F–C	.	.	.	C	.	.	.	.	.	C	N	.	C	.
52. Stoke Gabriel	B, M	Feb. 51	.	.	N	N	N	C	C	O	O	C	N	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
53. Brixham	RI, B	May 53	C	C	9·5	6·8	4·8	0·6	1·1	2·3	F	R	C	R	C	20%	A	30%	A	C	N	A	A	+	F	C	C	A	N
54. Torquay	RI, S	May 53	C	C	.	.	.	.	.	.	F	N	A	R	.	.	A	80%	A	C	.	A	A	+	F	C	C	C	N
55. Teignmouth	B, P, S	May 53	F	C	A	A	A	R	C	C	O	O	C	F	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
56. Dawlish	RF, P, S	May 53	F	C	5·5	3·5	1·5	N	2·0	5·0	O	.	C	R	.	.	C	50%	A	.	N	C	C	.	.	A	.	.	.
57. Starcross	B, P, M	July 54	.	.	0·005	N	N	2·5	3·5	4·5	O	.	F	F	.	.	N	N	F	.	.	.	.	.	C	N	F	C	.
58. Budleigh Salterton	RI, G	July 54	.	C	2·9	1·5	3·1	2·5	2·2	4·2	.	.	A	N	.	.	.	C	C	C	.	A	F	.	.	C	.	.	.
59. Sidmouth	RF, G, S	July 54	.	.	.	.	.	.	.	.	.	.	C	N	.	.	.	C	C	.	.	C	N	.	.	C	.	.	.
60. Branscombe	RF, G	July 54	F	C	C	O	N	C	A	A	.	.	F	N	.	.	.	F	C	.	.	C	C	.	.	F	.	F	.
61. Seaton	B, G	July 54	.	.	A	F	R	C	C	A	.	.	F	N	.	3%	.	30%	C	.	.	F	A	.	.	C	.	.	.
Dorset																													
62. Lyme Regis	RF, B, S, E	Aug. 52, Aug. 56	C	A	A	A	C	C	A	C	F	.	C	N	A	.	C	C	A	A	N	A	A	+	.	F	.	.	N
63. West Bay	RFI, G	July 54, April 57	.	.	3·5	3·5	0·8	0·5	1·0	1·3	.	.	A	N	.	0%	10%	70%	C	F	N	C	N	.	F	N	.	F	N
64. Portland Bill	RF	July 53	.	A	4·5	3·5	0·6	N	0·15	2·9	.	.	A	N	.	.	C	50%	A	.	.	C	N	.	.	A	.	.	N
64. Portland Bill	RF	April 56, April 57	.	.	1·2	1·2	.	N	0·34	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
65. Weymouth	S, P	July 53	.	.	O	.	.	A	.	.	.	.	R	F	.	0%	N	N	F	.	.	N	N	.	.	N	.	.	.
66. Osmington Mills	B, G	July 53	.	.	5·0	2·0	2·5	N	0·2	0·1	.	.	R	O	.	.	.	50%	C	.	.	A	N	.	.	F	.	.	.
67. Lulworth Cove	C, B, G	July 53	.	.	0·05	0·1	0·0003	0·02	0·05	0·001	.	.	N	R	.	.	.	20%	F	.	.	C	N	.	.	.	.	.	.
68. Kimmeridge	RF, B, G, E	July 53	.	C	4·0	5·0	3·0	0·01	0·01	0·2	.	.	N	F	.	.	.	25%	C	.	.	A	N	.	.	C	.	.	.
69. Peveril Pt., Swanage	RSI, B, S	June 49, July 53	C	A	0·16	0·2	0·0005	0·03	0·08	0·25	.	.	A–C	R	.	.	A	30%	A	.	.	C	N	.	.	C	.	.	N
70. Swanage Bay	B, G, S, P	June 49, July 53	.	.	.	.	.	.	.	.	.	.	N	C	.	6%	N	N	C	C	.	R	N	.	.	N	.	.	.
71. Studland	RF, S	June 49	.	.	N	N	N	A	.	.	.	.	C	C	.	.	.	.	.	.	.	N	N	.	.	R	.	.	.
72. Sandbanks	B, M, P	Mar. 48	.	.	N	N	N	A	.	.	C	C	F	F	.	.	.	.	.	.	.	.	.	.	.	N	.	.	.
Hampshire																													
73. Bournemouth	S, P	June 49	.	.	N	N	N	.	.	.	.	.	F	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
74. Hengistbury Head	B, G	July 49	.	.	R	N	N	F	F	C	O	.	O	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
75. Christchurch	G, M, P	July 49	.	.	N	N	N	A	A	A	.	.	N	A	.	1%	N	N	F	.	.	N	N	.	C	N	F	C	.
76. Mudeford	G, M, P	April 57	.	.	R	N	N	0·24	.	.	.	.	N	A	.	.	.	.	.	.	.	.	N	.	.	N	.	.	.
77. Southsea	B, S, P	Mar. 49, July 53, April 56	N	F	N	N	N	A	A	A	.	.	N	A	.	.	N	N	A	.	N	N	N	.	C	N	.	.	C

TABLE 7 *(continued)*

No.	Station	Description of shore	Dates of survey	*Anemonia sulcata*	*Actinia equina*	*Chthamalus stellatus*			*Balanus balanoides*			*Balanus crenatus*	*B. improvisus*	*B. perforatus*	*Elminius modestus*	*Verruca stroemia*	*Hemioniscus balani*	*Patella aspera*	*P. depressa*	*P. vulgata*	*Gibbula cineraria*	*G. pennanti*	*G. umbilicalis*	*Monodonta lineata*	*Calliostoma zizyphinum*	*Littorina littorea*	*L. neritoides*	*L. obtusata*	*L. saxatilis*	*Paracentrotus lividus*
						H.W.	M.T.	L.W.	H.W.	M.T.	L.W.																			
	Isle of Wight																													
78.	Alum Bay	RI, S	July 53	.	.	.	.	.	.	.	.	.	.	O	.	O	.	4%	12%	C	.	.	N	N	.	.	C	.	.	.
79.	Freshwater Bay	RFI	July 53	.	C	0·1	N	N	0·03	0·5	0·2	.	.	R	.	.	.	15%	30%	C	.	.	F	N	.	A	C	F	C	N
80.	Brook	RF, S	July 53	C	C	0·004	0·5	N	0·005	1·5	3·0	.	.	R	.	.	0%	.	40%	C	O	.	N	N	.	.	F	.	.	.
81.	Blackgang	RS, G	July 53	.	.	.	.	.	.	.	.	.	.	N	.	.	.	15%	30%	C	.	.	N	N	.	.	C	.	.	.
82.	St Catherine's Pt.	RS, B	July 53	.	F	0·003	N	N	0·2	0·4	1·0	.	.	N	.	.	.	3%	10%	C	.	.	N	N	.	.	F	.	A	.
83.	Ventnor	B, S	July 53	.	.	R	N	N	1·5	2·5	4·0	.	.	N	F	.	.	17%	3%	C	.	.	N	N	.	.	F	.	.	.
84.	Shanklin	S, P	July 53	.	.	N	N	N	A	A	A	C	.	N	.	.	.	N	N	C	.	.	N	N	.	.	N	.	.	.
85.	Sandown	S, P	July 53	.	R	N	N	N	4·0	6·0	6·0	.	.	N	.	.	.	N	N	O	.	.	N	N	.	.	F	.	.	.
86.	Lane end, Bembridge	RF, S, P	July 53	N	C	N	N	N	1·7	4·0	6·5	C	.	R	.	O	.	N	N	C	A	.	R	N	.	C	N	C	C	.
87.	Ryde	S, P	July 53	.	.	N	N	A	A	A	A	.	.	N	.	.	.	N	N	F	.	.	N	.	.	.	N	.	.	.
88.	Cowes	B, S, P	July 53	.	.	N	N	N	A	A	A	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
89.	Yarmouth	G, S, P	July 53	.	.	R	N	N	A	A	A	.	.	N	.	.	.	.	.	.	F	.	.	.	.	.	F	F	C	.
90.	Coldwell and Totland Bay	RFI, S	July 53	C	.	0·003	N	N	0·2	0·4	1·0	.	.	O	.	.	0%	10%	7%	.	C	.	R	N	.	.	C	C	C	.
	Sussex																													
91.	Selsey Bill	G, S, P	April 56	N	N	N	N	N	A	.	.	.	.	N	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
92.	Bognor Regis	S, P	June 53	N	.	N	N	N	A	A	A	.	N	N	C	.	.	N	N	F	.	.	N	N	.	F	N	.	F	.
93.	Littlehampton	S, P	June 53	N	.	N	N	N	C	C	C	.	O	N	A	.	0%	N	N	O	F	.	N	N	.	F	N	F	F	.
94.	Worthing	S, P	July 53	.	.	N	N	N	C	C	C	.	.	N	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
95.	Shoreham	B, M, P	July 53	.	.	N	N	N	A	A	A	C	C	N	C	.	2%	N	N	O	.	.	N	N	.	C	N	.	F	.
96.	Portslade	S, P	July 53	.	.	N	N	N	A	A	A	.	C	N	A	.	1%	.	.	.	.	.	N	N	.	.	.	.	.	.
97.	Brighton	G, P	July 53	.	.	N	N	N	C	C	C	.	.	N	F	.	.	.	.	.	.	.	.	.	.	.	N	.	C	.
98.	Black Rock, Brighton	C, G, P	Aug. 56	R	C	N	N	N	F	F	F	.	.	N	F	.	2%	N	N	C	O	.	N	N	.	F	N	F	C	.
99.	Rottingdean	C, G	Aug. 56	.	A	N	N	N	F	F	F	.	.	N	C	.	.	N	N	F	A	.	N	N	.	A	N	.	C	.
100.	Seaford	CI, B, G	July 53	.	.	N	N	N	A	A	A	.	.	N	O	.	.	N	N	.	O	.	N	N	.	.	N	.	C	.
101.	Eastbourne, Beachy Head	CI, B, G, S	July 53	.	.	N	N	N	C	C	C	.	.	N	C	O	.	N	N	F	.	.	N	N	.	.	N	.	C	N
102.	Bexhill	S, P	July 53	.	.	N	N	N	A	A	A	.	.	N	.	.	0%	N	N	F	O	.	N	N	.	F	N	.	.	.
103.	Hastings	S, P	July 53	.	.	N	N	N	A	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F	N	.	F	.
104.	Fairlight	RF, B, S	July 53	N	F	N	N	N	A	A	A	C	.	N	F	R	0%	N	N	C	N	.	N	N	.	A	N	F	C	.
	Kent																													
105.	Hythe	G, P	Aug. 56	.	.	N	N	N	C	C	C	.	.	N	O	.	.	N	N	N	.	.	N	N	.	N	N	N	F	.
106.	Folkestone	B, S	Aug. 56	.	.	N	N	N	A	A	A	C	.	N	F	.	0%	N	N	C	C	.	N	N	.	F	N	F	F	.
107.	Dover	B, S, P	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	C	.	0%	N	N	F	.	.	N	N	.	F	N	.	F	.
108.	Ramsgate	B, G	April 51	.	.	N	N	N	C	C	C	.	.	N	C	.	.	.	.	.	.	.	.	.	.	.	N	.	.	.
109.	North Foreland	CI, B, G	April 51	.	.	N	N	N	C	C	C	.	.	N	F	.	.	.	.	.	.	.	.	.	.	.	N	.	.	.
	Pas de Calais																													
110.	Calais	S, P	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	C	.	0%	.	.	.	.	.	N	N	.	.	R	.	C	.
111.	Cap Blanc Nez	CF	Sept. 54	.	.	N	N	N	F	F	F	.	.	.	R	.	.	N	N	N	.	.	N	N	.	.	N	N	N	N
112.	Audresselles	R, B, S	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	N	.	0%	N	N	C	.	.	R	N	.	.	N	.	C	.
113.	Wimereux	RI, G	Sept. 54	N	C	N	N	N	A	A	A	C	.	N	F	.	0%	N	N	A	O	.	C	N	.	.	N	C	.	.
114.	Boulogne	M, P	Sept. 54	.	.	.	.	.	.	.	.	.	C	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
115.	Berck-s.-mer	S	Sept. 54	.	.	.	.	.	.	.	.	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Seine Infèrieure																													
116.	Le Tréport	C, G	Sept. 54	.	C	.	.	.	.	.	.	.	.	N	F	.	.	.	.	.	.	.	N	N	.	C	N	.	.	.
117.	Dieppe	C, B, G	Sept. 54, Aug. 57	.	C	N	N	N	A	A	A	C	.	N	C	.	0%	N	N	C	O	N	F–C	N	.	C	N	F	C	.
118.	St Valéry-en-Caux	C, S	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	F	.	0%	.	N	F	.	.	O	N	.	.	N	F	.	.
119.	Fécamp	C, B, G	Sept. 54	.	.	N	N	N	C	C	C	.	.	N	F	.	0%	.	N	O	.	.	C	N	.	.	N	.	.	N
120.	Étretat	C, B, G	Sept. 54	.	.	N	N	N	A	A	A	.	.	.	.	.	0%	.	N	.	.	.	O	N	.	.	[illegible]	[illegible]	[illegible]	[illegible]

Calvados																														
122.	Honfleur	M, P	Sept. 54	.	.	N	N	.	C	C	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
123.	Deauville	C, B, S	Sept. 54	N	F	N	N	N	C	C	C	.	.	N	A	.	0%	.	.	.	.	.	N	.	.	C	N	.	C	.
124.	Cabourg	G, S	Sept. 54	.	.	.	N	.	.	N	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
125.	Longués	RF, B, G	Sept. 54	N	C	N	N	N	.	.	.	.	.	N	O	.	.	.	N	F	.	.	C	N	.	.	F	.	A	.
126.	Port en Bessin	CF, B, S	Aug. 57	.	.	.	.	.	.	.	.	.	.	.	C	.	2%	N	N	C	O	N	A	N	.	F	R	F	C	.
127.	West of Vierville-s.-mer	B, G	Sept. 54	N	C	N	N	N	A	A	A	.	.	N	F	.	.	.	N	F	.	.	O	.	.	.	N	C	A	.
128.	Pointe de Hoc	RS	Sept. 54, Aug. 57	N	A	N	N	N	A	A	A	.	.	N	C	.	0%	N	N	C	O	N	A	R	.	F	N	.	C	.
129.	Grandcamp-les-Bains	RF, B, G, P	Sept. 54, Aug. 57	R	F	.	.	.	.	.	.	F	.	N	C	.	0%	.	.	.	C	N	C	N	.	F	N	C	F	.
Manche																														
130.	Quineville	G, S	Sept. 54	.	.	N	N	N	C	C	C	.	.	.	A	.	.	N	N	N	.	.	N	N	.	.	N	N	F	.
131.	St Vaast-la-Hougue	B, M, P	Sept. 54, Aug. 57	O	A	N	N	N	C	C	C	.	.	R	C	.	6%	N	N	C	C	N	C	N	.	C	N	O	C	.
132.	Barfleur	RI, S, M	Sept. 54	C	.	.	.	.	.	.	.	.	.	N	F	.	0%	.	N	O	C	C	C	C	O	.	.	.	.	.
133.	Pte. de Barfleur	RF, E	Sept. 54, Aug. 57	C	A	N	N	N	A	A	A	.	.	C	.	.	0%	C	23%	A	F	F	C	R	O	.	R	C	C	N
134.	Cap Lévy	RF, B	Sept. 54, Aug. 57	A	F	N	N	N	A	A	A	C	.	C	.	.	4%	20%	35%	A	C	A	C	R	+	.	O	F	F	.
135.	Bretteville	B, G	Sept. 54	.	C	N	N	N	A	A	A	.	.	R	O	.	.	C	40%	C	.	.	C	R	.	.	.	.	.	.
136.	Cherbourg Harbour	M, P	Sept. 54	.	.	N	N	N	C	C	C	.	.	.	C	.	50%	.	.	.	.	.	.	.	.	.	N	.	.	.
137.	Nacqueville	RI, S	Sept. 54	C	C	.	N	N	.	C	A	.	.	O	O	.	10%	F	3%	C	F	.	C	R	.	.	F	.	C	.
138.	Rocher du Castel Vendon	RS	Sept. 54	.	.	N	N	N	A	A	A	.	.	.	.	.	.	.	F	C	.	.	C	C	.	.	F	.	.	N
139.	Omonville-la-Rogue	R, B, G	Sept. 54	.	C	N	N	N	A	A	A	.	.	.	R	.	.	.	60%	C	.	.	.	.	.	.	C	.	.	.
140.	Cap de la Hague	RF, E	Sept. 54	C	.	N	N	N	A	A	A	.	.	.	O	.	.	.	C	C	.	.	C	A	.	.	F	.	.	N
141.	Vauville	R, S	Sept. 54	.	.	0·0005	N	N	A	A	A	.	.	.	O	.	10%	.	40%	C	.	.	.	C	.	.	C	.	.	.
142.	Cap de Carteret	RI, S	Sept. 54	N	A	0·0005	N	N	5·6	4·5	5·0	.	.	O	.	.	.	C	.	.	.	.	O	O	.	F	C	.	C	N
143.	Carteret	S, P	Sept. 54	.	.	0·01	.	.	3·8	.	.	.	.	.	.	.	0%	.	20%	C	.	.	.	.	.	.	C	.	.	.
144.	Gouville	RI, G, S, E	Sept. 54	F	C	N	N	0·001	3·5	3·5	4·0	.	.	N	R	.	20%	O	N	F	F	.	C	C	.	A	N	F	F	.
145.	Granville	R, G, S	Sept. 54, Aug. 57	C	A	1·5	N	N	2·0	2·9	3·3	.	.	F–C	.	.	4%	C	N	A	.	C	A	C	.	F	F	C	C	.
146.	Carolles	RF, B, S	Aug. 57	F	A	0·25	N	N	C	5·2	C	F	.	R	N	.	0%	N	N	C	O	N	A	A	.	C	N	.	F	.
147.	St Jean le Thomas	B, S	Sept. 54	.	.	0·5	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F	R	F	A	.
148.	Mont St Michel	RS, M	Aug. 57	.	.	0·002	.	.	0·05	.	.	.	.	.	.	.	3%	.	.	.	.	.	.	.	.	.	.	.	.	.
Channel Islands: Alderney																														
149.	Clonque Fort (sheltered)	RI, B, S, E	Sept. 56	A	A	0·06	0·07	0·02	0·35	2·7	2·5	.	.	.	N	.	.	.	.	.	C	A	A	A	.	N	A	.	.	N
150.	Clonque Fort (outer reef)	RS	Sept. 56	.	A	0·09	0·25	0·5	1·9	1·8	1·8	.	.	.	N	A	0%	A	65%	A	.	.	.	.	+	.	.	.	.	N
151.	Pt. Grosnez	RIS	Sept. 56	A	A	0·03	0·23	0·02	2·2	2·7	3·1	.	.	A	N	.	.	.	.	.	.	A	A	A	.	.	.	.	.	.
152.	Braye Harbour and beach	B, S, P	Sept. 56	.	.	0·025	.	.	.	.	.	.	.	F	N	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.
153.	Pt. Quesnard	RSF, E	Sept. 56	.	.	0·004	0·0002	N	3·6	2·8	2·8	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
154.	Longy Bay	B, G, S	Sept. 56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	A	.	N	.	.	.	.
155.	Telegraph Bay	RI, B, S	Sept. 56	A	A	0·025	0·01	0·005	1·8	4·0	2·4	.	.	A	.	.	10%	.	.	.	A	A	A	A	.	.	A	.	.	N
Guernsey																														
156.	St Peter Port	RI	Sept. 56	.	C	0·0004	N	N	A	A	A	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
157.	Bordeaux Harbour	B, S	Sept. 56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	C	C	.	.	.	.	.	.
158.	Fort Doyle	RI	Sept. 56	A	A	0·01	0·02	N	3·0	3·2	2·5	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
159.	Grand Camp	RI, B, E	Sept. 56	.	.	0·05	0·06	0·24	1·6	2·7	1·8	.	.	A	.	.	35%	.	60%	.	C	C	C	C	.	.	.	.	.	N
160.	Isle Lihou	RI, B, E	Sept. 56	C	C	0·04	0·05	0·08	2·5	3·2	1·9	.	.	.	.	.	44%	.	85%	.	.	A	A	A	.	.	A	.	.	N
161.	La Moye Pt.	RS	Sept. 56	.	A	0·36	0·30	0·005	2·2	3·5	1·6	.	.	A	.	.	.	.	90%	.	.	.	.	.	.	.	.	.	.	N
162.	Jerbourg Pt.	RS	Sept. 56	A	A	0·015	N	N	3·3	4·4	2·0	.	.	A	.	.	.	.	.	.	A	A	A	A	.	.	A	.	.	N
Jersey																														
163.	Montorgueil Castle	RS, G, S	Sept. 56	A	A	0·01	0·001	N	2·8	3·6	1·6	.	.	R	.	.	.	.	.	.	.	C	A	A	.	F	.	.	.	N
164.	Elizabeth Castle	RI, S, E	Sept. 56	A	A	0·02	N	N	4·8	4·8	4·9	.	.	N	.	.	.	.	.	.	A	C	A	A	.	.	.	.	.	.
165.	Fremont Pt.	RI	Sept. 56	.	.	0·045	0·001	.	2·7	2·2	.	.	.	N	.	.	25%	.	90%	.	.	O	.	A	.	.	A	.	.	.
166.	Plemont Pt.	RS	Sept. 56	.	.	0·02	0·04	N	3·4	6·0	4·8	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
167.	La Corbière	RI, B, E	Sept. 56	A	A	0·007	0·015	N	7·0	2·4	4·0	.	.	.	.	.	0%	.	.	.	.	C	.	.	+	.	A	.	.	N
Ille-et-Vilaine																														
168.	Cancale	RS, B	Sept. 54, Aug. 57	A	C	1·9	2·8	.	0·9	1·4	.	.	.	.	.	.	20%	.	38%	C	.	C	C	A	+	C	R	.	C	.
169.	Pte. du Grouin	RS	Aug. 57	A	F	2·8	2·0	0·7	0·3	1·2	2·2	.	.	C	N	.	.	C	90%	F	.	C	F	C	+	O	R	.	A	N
170.	Rothéneuf	RS	Sept. 54	C	C	4·1	1·8	.	0·3	2·4	.	.	.	.	.	.	.	F	70%	A	.	.	C	C	.	.	R	.	A	.
171.	St Malo	RI, S, B	Aug. 57	A	.	2·3	1·7	.	0·5	1·8	.	.	.	C	.	.	.	C	90%	C	.	A	A	C	+	C	O	A	C	.

TABLE 7 (*continued*)

No.	Station	Description of shore	Dates of survey	*Anemonia sulcata*	*Actinia equina*	*Chthamalus stellatus*			*Balanus balanoides*			*Balanus crenatus*	*B. improvisus*	*B. perforatus*	*Elminius modestus*	*Verruca stroemia*	*Hemioniscus balani*	*Patella aspera*	*P. depressa*	*P. vulgata*	*Gibbula cineraria*	*G. pennanti*	*G. umbilicalis*	*Monodonta lineata*	*Calliostoma zizyphinum*	*Littorina littorea*	*L. neritoides*	*L. obtusata*	*L. saxatilis*	*Paracentrotus lividus*
						H.W.	M.T.	L.W.	H.W.	M.T.	L.W.																			
Côtes-du-Nord																														
172.	Dinard	RI, S	Sept. 54, Aug. 57	C	F	2·7	2·5	0·6	1·5	2·0	2·9	.	.	C	N	.	.	C	80 %	C	.	C	A	C	.	.	C	.	A	.
173.	Notre-Dame-du-Guildo	S, M, P	Sept. 54	.	.	C	O	.	C	A	.	.	.	.	O	.	30 %	.	.	.	.	.	.	.	.	.	.	.	.	.
174.	St Cast	RI, E	Aug. 57	A	O	4·1	2·5	1·5	R	1·8	3·1	.	.	A	N	.	17 %	C	67 %	C	.	C	A	C	+	O	O	.	C	+
175.	Cap Fréhel	RS	Sept. 54, Aug. 57	R	.	2·9	2·5	1·5	1·0	2·5	2·4	.	.	A	N	.	.	C	70 %	A	C	F	C	O	O	F	C	O	A	+
176.	Cap d'Erquy	RS, B	Aug. 57	O	O	2·7	1·2	3·3	N	1·4	0·1	.	.	A	N	.	.	.	75 %	C	.	F	C–A	A	.	C	O	C	A	+
177.	Pte. de Pléneuf	RSI	Sept. 54	C	C	6·0	3·6	2·5	0·1	1·8	2·3	.	.	C	N	.	.	.	80 %	C	.	C	C	C	.	.	C	.	C	N
178.	le Val André	M, P	Sept. 54	.	.	0·005	.	.	.	.	.	.	.	.	N	.	10 %	.	.	.	.	.	.	O	.	.	.	.	.	.
179.	Pte. de Cesson	G, M, P	Sept. 54	.	.	0·01	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	O	.	.	F	.
180.	Pte. du Roselier	RS, G	Sept. 54	.	C	4·5	2·1	1·1	0·2	1·5	2·3	.	.	.	.	.	.	.	90 %	A	.	.	C	O	.	.	F	.	C	.
181.	Pte. de Pordic	RS, G	Aug. 57	O	A	3·4	2·8	1·0	0·2	3·4	0·5	.	.	C	N	.	.	A	91 %	C	C	N	A	C	.	A	F	.	C	N
182.	Binic	P, M	Aug. 57	.	.	.	2·0	.	.	2·0	.	.	.	.	R	.	26 %	N	O	A	.	.	.	.	.	.	.	.	.	.
183.	St Quay—Portrieux	RSI, S	Sept. 54, Aug. 57	F	C	3·5	1·6	1·0	1·6	3·4	2·5	.	.	A	N	.	38 %	F	81 %	A	C	C	A	C	.	C	O	.	A	N
184.	Lèzardrieux	B, M, P	Sept. 54	.	.	O	.	.	C	.	.	.	.	.	N	.	.	.	.	C	.	.	C	.	.	.	.	.	C	.
185.	Sillon de Talbert	RI, G, E	Sept. 54	.	.	2·2	0·5	.	1·0	1·5	.	.	.	.	N	.	.	.	10 %	.	C	C	O	A	.	.	C	.	F	N
186.	Tréguier	B, M, P	Sept. 54	.	.	1·5	2·0	0·3	0·2	1·2	2·8	F	C	C	C	.	11 %	.	.	C	.	.	A	C	.	.	.	.	.	N
187.	Trestrignel	RI, B, S	Aug. 57	A	A	2·2	2·7	.	0·3	1·3	.	.	.	.	.	.	.	C	25 %	A	.	.	C	A	.	.	C	O	A	.
188.	Trégastel-Plage	RI, B, S	Sept. 54	C	C	2·0	2·0	2·0	.	.	.	.	.	O	.	.	.	.	5 %	C	C	.	C	C	C	F	C	.	.	N
189.	Pte. de Bihit	RI B, S	Aug. 57	.	.	.	2·2	1·5	.	1·9	1·6	.	.	O	.	.	.	N	0 %	A	.	A	C	C	.	A	R	C	C	.
190.	Pte. de Dourvin	RI, E	Aug. 57	C	O	A	.	C	N	.	O	C	.	C	.	O	.	C	70 %	C	A	A	A	A	.	.	C	.	C	+
191.	Pte. de l'Armorique	RI	Sept. 54	C	C	.	.	.	.	.	.	.	.	O	.	.	.	.	.	.	.	.	C	C	.	F	A	.	F	+
Finistère																														
192.	Pte. de Locquirec	RF, S	Aug. 57	C	C	4·1	3·7	1·6	N	0·05	0·1	.	.	A	.	.	.	C	67 %	C	.	A	C	A	.	A	C	.	F	+
193.	Primel-Trégastel	R, B	Sept. 54	.	.	.	.	.	O	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	N
194.	Pte. de Primel	RSI	Sept. 54	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	60 %	C	C	.	C	A	.	R	A	.	F	+
195.	Morlaix	M, P	Sept. 54	.	.	.	.	.	.	.	.	.	C	F	O	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.
196.	Penzé	P, M	Aug. 57	.	.	.	.	.	.	.	.	.	.	.	A	.	33 %	.	.	.	.	.	.	.	.	.	.	.	.	.
197.	Pte. de Bloscon, Roscoff	RI, B	Aug. 57	F	O	3·2	2·5	1·8	R	0·2	0·002	.	.	A	.	.	.	C	17 %	C	C	A	A	A	O	O	C	C	A	+
198.	Grève de Poulfoën	RF, B, S, E	Aug. 57	F	.	0·8	3·1	0·9	0·3	0·15	0·01	.	.	C	.	.	0 %	A	73 %	C	C	A	A	F	.	.	C	.	A	.
199.	Pte. de Pontusval	R, B, S	Sept. 54, Aug. 57	R	.	C	0·2	.	O	0·5	.	.	.	C	R	.	.	.	60 %	.	A	C	C	O	.	.	F	C	C	N
200.	Grèves du Vougot	RFI, S, E	Sept. 54 Aug. 57	C	.	A	.	.	N	.	.	.	.	.	O	.	.	.	60 %	C	C	F	C	R	+	.	C	A	C	N
201.	Grève de Lilia	RS, B	Aug. 57	O	F	1·2	.	.	0·3	.	.	.	.	.	N	.	.	.	.	O	.	O	C	.	.	F	F	A	A	.
202.	L'Aber Wrac'h	B, M, P	Sept. 54	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
203.	Tréglonou	B, S, P	Sept. 54	.	.	.	.	.	O	.	.	.	F	O	C	.	5 %	.	.	.	.	.	.	.	.	.	.	.	.	.
204.	Trémazan	RSI, B	Sept. 54, Aug. 57	F	C	1·8	3·0	2·1	N	N	0·08	.	.	R	N	.	.	C	R	C	C	A	C	R	+	F	A	C	C	N
205.	Melon	RF, B	Aug. 57	C	F	1·9	A	2·2	0·02	O	0·04	.	.	N	N	.	5 %	C	R	C	O	F	A	.	+	.	C	A	C	N
206.	L'Aber Ildut	B, M	Aug. 57	.	.	.	O	.	N	N	N	.	.	.	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
207.	Le Conquet	RSI, B	Sept. 54, Aug. 57	A	C	4·3	4·9	2·2	R	0·03	0·02	C	.	A	R	.	0 %	C	60 %	C	O	A	A	C	.	.	A	.	F	.
Ile d'Ouessant																														
208.	Baie du Stiff	RI, B, S	Aug. 57	O	A	1·8	2·5	0·4	0·2	0·2	0·0005	.	.	R	N	.	0 %	C	N	A	O	C	C	N	.	O	C	.	A	.
209.	Pte. du Stiff	RS, I	Aug. 57	.	A	1·0	2·0	.	N	0·05	.	.	.	R	N	.	.	C	N	F	.	.	.	.	.	.	A	.	C	.
210.	S.E. point of island	RI, B	Aug. 57	C	A	1·4	R	O	N	N	N	.	.	.	N	.	.	C	R	C	.	.	C	O	.	.	C	.	.	.
211.	Baie de Lampaul	RS	Aug. 57	O	C	C	C	C	N	0·0001	N	.	.	R	N	.	.	C	3 %	C	C	N	A	R	+	.	.	.	.	.
212.	Lampaul	M, P	Aug. 57	.	.	0·3	.	.	.	0·02	.	.	.	.	O	.	.	.	.	.	C	N	C	N	.	.	.	C	C	.
213.	Pte. de Pern	RI, E	Aug. 57	O	A	1·1	0·9	0·8	N	N	0·01	.	.	C	N	.	.	O	R	A	O	O	O	N	.	N	C	N	C	.
214.	Phare de Créac'h	RS	Aug. 57	.	C	.	.	.	N	N	N	.	.	N	N	.	.	A	R	A	.	.	.	.	.	.	C	.	.	+

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STUDIES ON MARINE FLAGELLATES

IV. MORPHOLOGY AND MICROANATOMY OF A NEW SPECIES OF *CHRYSOCHROMULINA*

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(With total of 37 Figures in text and on Plates I–X)

CONTENTS

	PAGE
Introduction	209
Materials and methods	210
Morphology of the cell as a whole	211
The scale characters	213
Structure of the flagella and the haptonema	215
Contents of the body as seen with the light microscope	217
Contents of the body as seen with the electron microscope	219
Behaviour of the living cell and reproduction	222
Discussion	223
Formal taxonomic diagnosis	225
Chrysochromulina chiton sp.nov. (Plymouth no. 146)	225
Acknowledgements	227
Summary	227
References	227

INTRODUCTION

In our previous descriptions of new species of flagellates provisionally referred to the genus *Chrysochromulina* (Parke, Manton & Clarke, 1955, 1956) the electron microscope has been used to amplify knowledge of the external morphology but not of the internal structure. This limitation was known to be temporary, but considerable effort has had to be expended on perfecting methods of handling these very delicate organisms before it could be overcome. That to some extent this has now been achieved is due to two independent circumstances, on the one hand a fairly large and relatively resistant organism with some specially favourable features about its external appendages, and on the other hand the opportunity of examining good sections with a more powerful microscope than that normally available to us in Leeds.

The optical requirements for the electron microscopy of thin sections of biological material are far more stringent than for shadow-cast whole mounts and without the RCA microscope at the Rockefeller Institute, or some equivalent instrument, the sections reproduced here could not have been effectively studied. That this microscope was made available to one of us (I. M.) during a 2 weeks' visit to New York at Christmas 1956 is a matter for very lively personal gratitude to Dr K. R. Porter and other members of the staff of the Cytology Section who were at hand at a holiday period. As a result, although several other problems were studied during this visit, the work on the new flagellate advanced sufficiently far to reach a state suitable for publication with only 4 days' further work on a high-resolution microscope in England (the Siemens microscope at the Aeon Laboratories at Egham, Surrey).

It should perhaps be explained, however, that in a study of this kind, even without the present limitation of access to suitable microscopes, completeness in the description of fine structure is rarely a practicable objective at the first attempt. With every new type of cell it is a sufficient undertaking to identify the various organs present and determine their salient structural features. This is all that we are attempting here, but among the organs known to be present two stand out as of special interest in the present context, namely, the scales and the haptonema. Without the sections of scales the taxonomic description of this particular species could not have been completed, and special attention has necessarily been devoted to them for this purpose. The haptonema, on the other hand, is an organ of general interest which characterizes the whole genus but about which nothing is known anatomically. Though much still remains unknown we have succeeded in revealing at least some of the more obvious structural differences which distinguish a haptonema from the flagella with which it was formerly confused (Lackey, 1939), and since it will in any case be necessary to examine this unfamiliar organ in more than one species we may hope for future opportunities to extend our present limited knowledge. The other body organs have been examined only incidentally. Most, and probably all, of those detected with the light microscope have been found with the electron microscope and a few others have been added. But in all, our study of structure has been limited to the necessary minimum required for identification of the organ, since space as well as time would have precluded more detailed analysis.

MATERIALS AND METHODS

The material came from three separate isolates originally numbered 146, 149 and 150 in the Plymouth collection. All three have been used for external morphology, but only culture 146 was embedded. As on previous occasions each culture was unialgal though not bacteria-free.

The methods used for visual microscopy and for external morphology with the electron microscope have been exactly as in our previous papers. They are described in Parke *et al.* (1955), and therefore the only new details to mention are those concerned with the production of thin sections for the electron microscope.

Before embedding it is necessary to concentrate the cells by 10–15 min centrifugation at a speed which has to be determined by experiment. The test for successful centrifugation is that there should be sufficient cells in the concentrate and that these should still be swimming when examined under the light microscope; it is not necessary to remove all cells from the supernatant. The fixative (1 % osmium tetroxide buffered to pH 7 with acetate veronal in distilled water) is then poured on after removal of the supernatant and the tube is left undisturbed in a domestic refrigerator for 1 h. Alternatively, the concentrated cells may be sucked up with a pipette and squirted into the fixative before being again spun down for ease of handling.

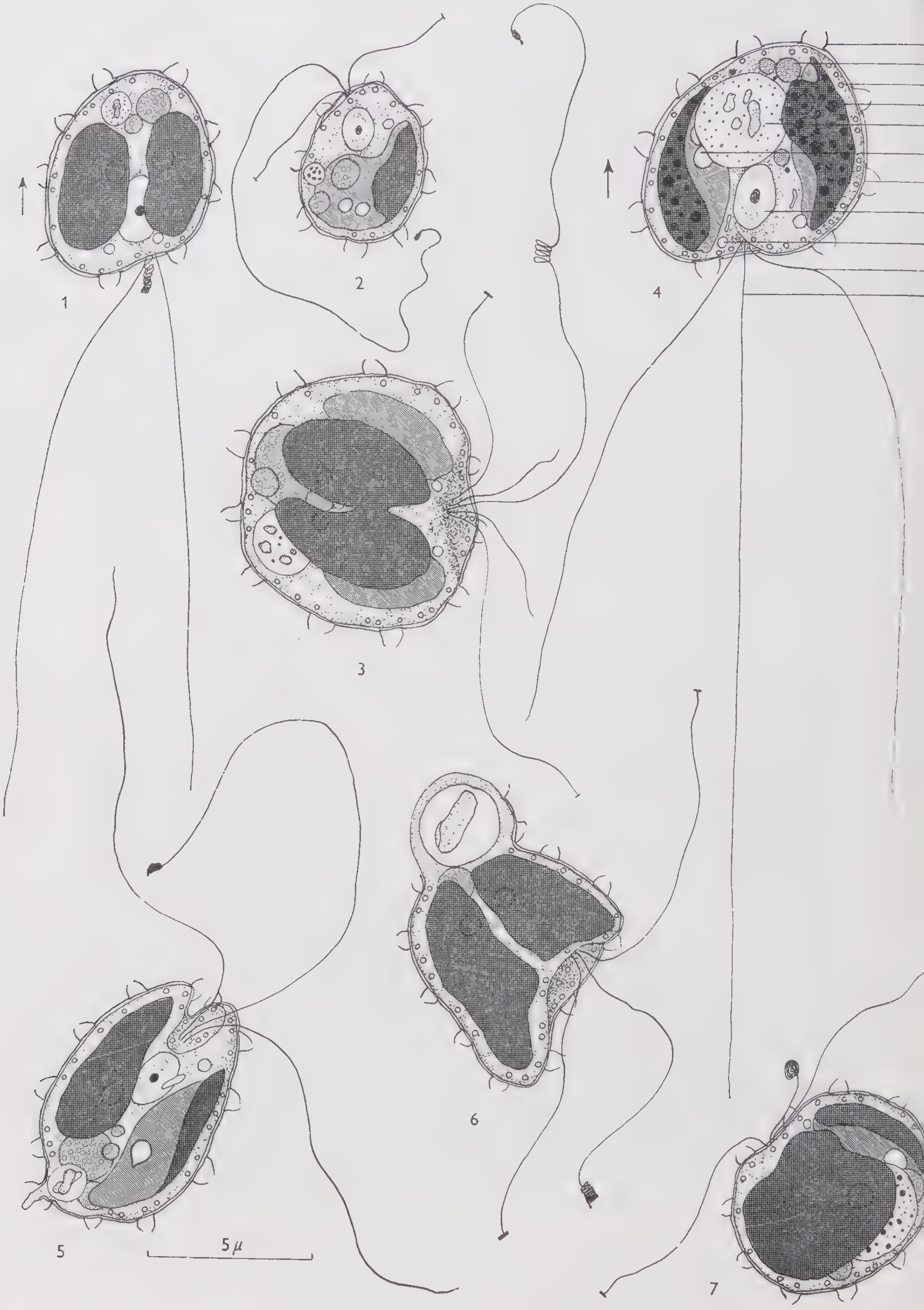
The procedure for washing, dehydration and embedding in *n*-butyl methacrylate is standard. It is probably enough to say that the fixative is washed out by two rapid changes of buffer and the dehydration is by means of graded alcohols—30, 50, 70, 90 % (two changes), absolute (two changes)—each applied for a quarter of an hour. After this the material is transferred by stages to the destabilized monomer which is polymerized by the aid of the commercial catalyst Luperco in an oven at 48° C.

The sections were cut on a Porter-Blum Serval microtome in the Leeds Botany Department using a glass knife.

MORPHOLOGY OF THE CELL AS A WHOLE

The essential characteristics of the genus *Chrysochromulina* as at present construed have been discussed in previous papers (Parke *et al.*, 1955, 1956), and therefore we propose to give close attention in this section only to those details by which our new species is distinguishable from others.

The general shape of the cell is best illustrated in Figs. 1–7, since the photographs assembled in Pl. I are necessarily based on dried material. In an actively growing culture 75 % of the cells are from 5 to 8 μ diameter, while 8 % are between 4 and 5 μ; the remaining 17 % are incipient fission stages from 8 to 10 μ. These dimensions are thus somewhat larger than those of *C. kappa*, *C. minor* and *C. brevifilum*, though slightly smaller than those of *C. ericina*, *C. ephippium* and *C. alifera*. The two flagella are equal in length in only about 25 % of the cells, the remainder having them subequal; 50 % of these show a difference of 1–2 μ, 45 % show a difference of 2–4 μ and 5 % a difference of 5–6 μ between the two flagella of an individual. This degree of asymmetry is slightly greater than that described for *C. alifera* though the flagellar movement appears to be homodynamic. The haptonema is relatively short, being only a little longer than the flagella (cf. Pl. I). This detail recalls *C. ericina* and, as in that species, the attachment of the three appendages to the body is slightly 'off centre' in a depression at the pole which commonly faces



Figs. 1–7. *Chrysochromulina chiton* sp.nov. (× 5000)

backwards during swimming. Further details regarding the behaviour of the cell in life will be found on p. 222.

In spite of a superficial resemblance to some of our other species in various details of relative dimensions our new species is sharply distinguished from everything that we have previously described by its very remarkable and large scales. These are so big (up to nearly 3 μ in diameter) that they can be seen individually with the light microscope on a dried preparation (cf. Fig. 10, Pl. I), but even without drying they are liable to become detached from the surface of the body in sheets which stain readily with cresyl blue and are then clearly visible. In section they are equally distinctive in ways which will be described below. For these reasons we propose to name our new species *C. chiton* to draw attention to the remarkable scale characters.

THE SCALE CHARACTERS

The large size and spectacular appearance of the scales can be seen in Fig. 11, Pl. I, and in Pls. II and III. They are manifestly of two kinds, each with characteristic differences in the appearance of the two faces. There are smaller, roundish or oval scales, each with a rim and a radiating pattern of ridges on one face which is absent from the other (see specially Figs. 16 and 17). These represent a general type of scale which we have encountered not uncommonly (1955, 1956), often in association with others of markedly different appearance. The second type of scale and the one characteristic of our new species has not previously been encountered. These scales are much larger and manifestly bipartite, having a wide rim delimited from an oval centre by a ridge which suggests that these two parts cannot have been in the same plane in life, although in the flattened condition produced by drying it is not possible to deduce what their mutual relations may have been. Unconsciously one is influenced by their astonishing resemblance to straw hats. The pattern of

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Legends to Text-figs. 1–7

Chrysochromulina chiton sp.nov. (× 5000)

Fig. 1. Individual swimming with flagella and haptonema behind body in position characteristic for the species during rapid swimming.

Fig. 2. Anchored individual with one chromatophore; haptonema nearly fully extended but bent.

Fig. 3. Early fission stage: four flagella, one haptonema, chromatophores dividing.

Fig. 4. Individual gliding without rotating, with flagella and haptonema behind body in position characteristic for the species during gliding movement. *c*, chromatophore containing saturated lipoid globules stained by Sudan Black; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid; *s*, smaller plate-shaped scale; *ss*, larger saucer-shaped scale; *v*, vacuole containing ingested bacteria; *x*, mitochondrion?

Fig. 5. Individual swinging round on partly extended, bent, anchored haptonema; recently ingested bacteria in vacuole at non-flagellar pole.

Fig. 6. Anchored individual with haptonema coiled at distal end; recently ingested *Monas* cell in vacuole at non-flagellar pole.

Fig. 7. Anchored individual with haptonema coiled; large vacuole containing granules showing Brownian movement near non-flagellar pole.

sculpturing which is partly responsible for this illusion is present on one face only, the other face being smooth (compare S1 with S2 in Fig. 15, Pl. II).

The true shape of both types of scale is at once made clear from a section, e.g. Fig. 30, Pl. IX. The large scales are not hat-shaped but saucer-shaped, and the small scales are plate-like with a rim sharply flexed towards the upper surface. Both kinds of scale become detached from the cells so easily that these are commonly naked when sectioned though scales may litter the field cut at all angles and without orientation to a surface (Pl. IV, etc.). The two characteristic types of profile are easily picked out. Occasionally, however, a favourable cell may retain its scales in position and two are illustrated here (Pl. VIII and Fig. 29, Pl. IX) from each of which the arrangement of the scales on the cell surface is obvious. They form a single layer with the two scale types so arranged that the small scales alternate with the large ones, occupying the interstices between them. In surface view this must mean that each large scale is surrounded by a ring of 5–6 small scales in a manner which is partly retained in the detached mat illustrated in Pl. III.

Apart from the evidence on scale arrangement, the cells of Figs. 28 and 29, Pls. VIII and IX, are important for providing information regarding the relative positions of the patterned versus patternless scale faces with respect to the cell surface. In both cases the scale shape in section combined with the information from shadowcast material makes it clear that the patterned surface lies against the cell body and the relatively patternless surface outwards. This is in exact agreement with the position deduced from the evidence of the spined scales in *C. ephippium* (1956, p. 402).

A fuller interpretation of the nature of the patterning on the scale faces cannot be arrived at without knowledge of their mode of growth. There is, however, no doubt that, at least for the larger scales, there are two quite distinct layers of material present which in section can be seen separately in the angle where the rim joins the central plate (Pl. VIII). Though the

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Explanation of Plates I–II

Chrysochromulina chiton sp.nov.

I

Figs. 8–10. Three dried cells photographed with the light microscope to show the flagella and haptonemata extended; detached scales visible near arrows in Fig. 10. × 1000.

Fig. 11. Low-power electron micrograph (M489.10) of a cell with a coiled haptonema; the body scales very conspicuous. × 3000.

Fig. 12. Similar but with haptonema extended and scales not conspicuous. M380.24, × 3000.

Fig. 13. Tip of the haptonema and one flagellum of Fig. 12 more highly magnified. M 380.15, × 10,000.

II

Fig. 14. Tip of a haptonema to show apical swelling and sheath. M 480.6, × 18,000.

Fig. 15. A cell with scales, S1 showing smooth outer face, S 2 showing patterned lower face. M 301.6, × 15,000.

Fig. 16. A field of detached scales more highly resolved. Mid 502.5, × 30,000.

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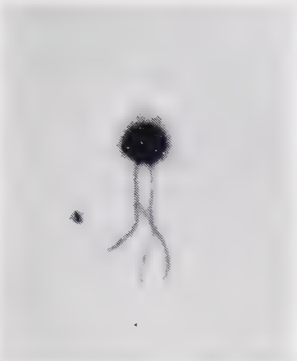

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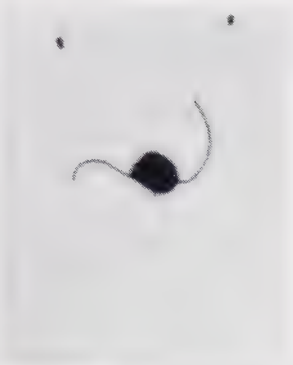

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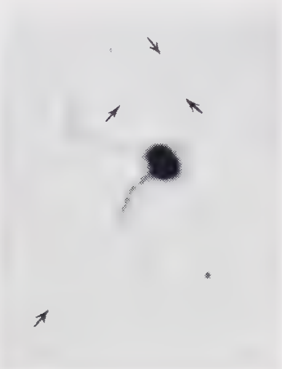

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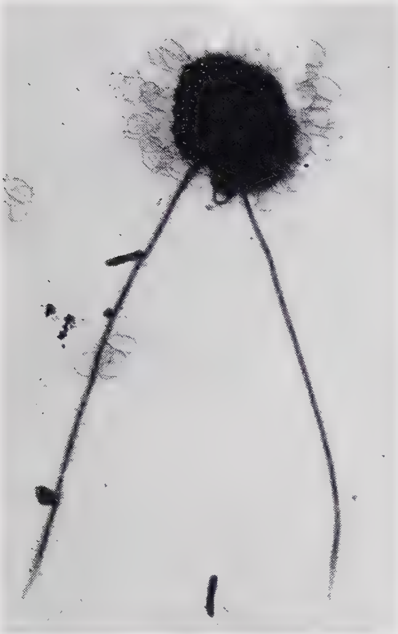

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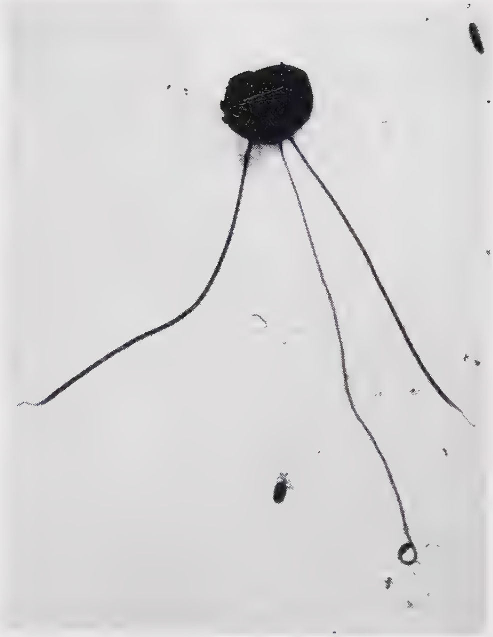

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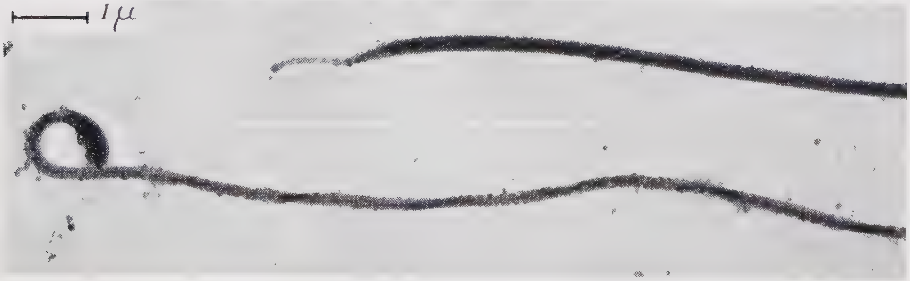

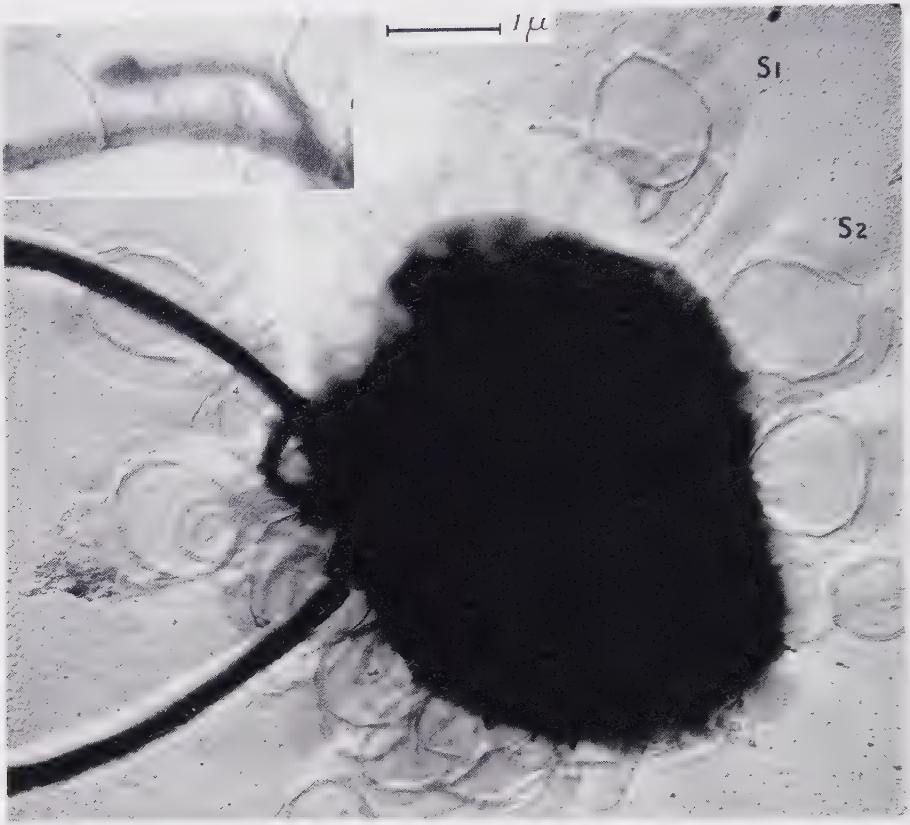

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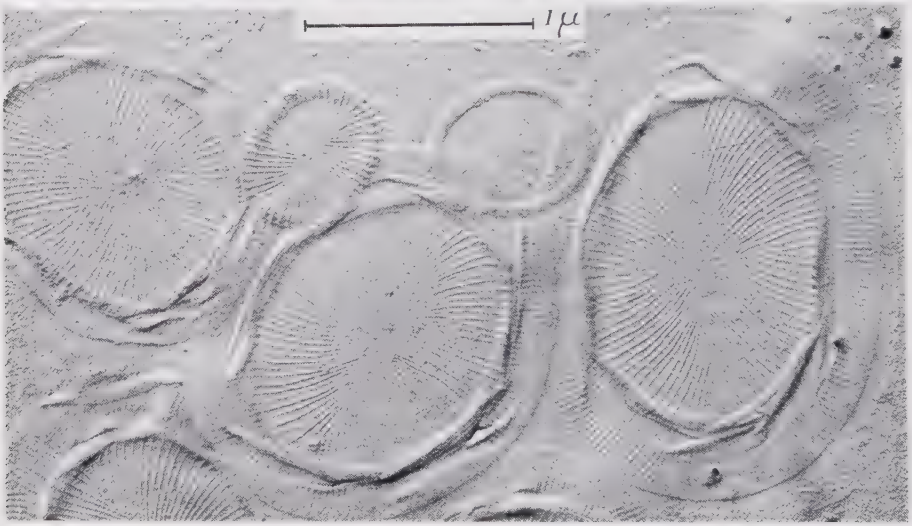

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sculpturing is confined to the lower layer, it is probable that this layer is actually compound since our best resolved pictures of the patterned surface, Fig. 16, Pl. II, give a strong suggestion of interwoven fibres rather than sculpturing in the strict sense.

STRUCTURE OF THE FLAGELLA AND THE HAPTONEMA

The two types of filiform appendages are visible in Pl. I and part of Pl. II. The club-shaped tip of the haptonema contrasting with the attenuated knobbed apices of the flagella (Fig. 13) is a familiar feature from other species. That the axis of the haptonema is narrower than that of a flagellum is also familiar, as is the occasional appearance as of a loose sheath covering the haptonema in favourable preparations (Fig. 14).

The internal structure of flagella is now so well known that they can be recognized at sight in almost any type of section. In Fig. 30, Pl. IX, for example, there is a good transverse section showing the outer membrane, the ring of nine strands and the central pair, though the latter are not separately resolved. Other views will be found in several of the micrographs, but special attention can usefully be given to the one on the right of Fig. 21, Pl. V, since this, though somewhat flattened, shows the two central strands clearly and also traces of the split in some of the peripheral strands.

The other objects contained in the various micrographs on Pl. V are almost all parts of coiled haptonemata cut in various directions. In studying the internal structure of the haptonema the first and major difficulty is identifying it in section, since its attachment to the body cannot usually be traced. The coiled nature of the haptonema, however, in this species made identification possible, since in section the haptonema gave clusters of small profiles with an opaque micro-structure which could be distinguished from other small circular profiles (flagella, bacteria, and the debris from broken cells) which crowd the field. The clusters of small profiles are a sure indication of the presence of a haptonema once the general characteristics of flagella and bacteria, both of which are larger than haptonemata in section, have been recognized. A characteristic field containing a haptonema is reproduced at a low magnification in Pl. IV, and more highly magnified parts of four different specimens are included in Pl. V.

A very common fixation artifact which is unavoidably represented in parts of each of the four haptonemata reproduced in Pl. V is 'blistering'. This is almost certainly due to the presence of osmotically active materials beneath a relatively impermeable membrane, and the local distension of this membrane may cause not only a blister-like swelling of the surface but also distortion of the more resistant internal parts of the organ. In flagella, where this artifact is well known, the axis may become bent within the blister so that certain planes of section will suggest the presence of two or more axes within a common skin. Something similar is occurring in parts of all the haptonemata illustrated in Pl. V and therefore not every profile can be interpreted as normal. Since, however, all sections in any one cluster belong to one and the same organ it is generally possible to distinguish undistorted sections as such when the plane of cutting is favourable.

The best transverse sections in Figs. 19–21 are indicated by arrows, and Figs. 21 *a* and *b* represent two successive sections reproduced side by side. Some other profiles in the various figures are oblique or longitudinal. Wherever the orientation is adequate, however, there is complete agreement in the numerical details displayed. In contrast to the flagella a haptonema possesses at least three concentric membranes surrounding a ring of seven strands, and the centre of the organ seems to be hollow. The sectional outline is probably circular except where flattening by pressure from the knife has occurred in cutting.

In many specimens, notably that of Fig. 20, the ring of seven strands (or tubes) is closely pressed to the inner side of the innermost membrane, but they are definitely not part of this membrane since they can become detached from it if blistering extends to the interior of the whole organ (Fig. 22). The outermost membrane in this specimen is greatly distended and within it the axis of the organ has been transversed three times. Two of these axial sections

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Explanation of Plates III–VI

Chrysochromulina chiton sp.nov.

III

Fig. 17. A field of scales, reversed print. Mid 301.18, × 20,000.

IV

Fig. 18. Section of a field containing a haptonema (arrow) between cells and debris. S 37, × 12,500.

V

Fig. 19. Another section of the haptonema contained in Pl. IV at a different angle on the page; parts of eight profiles through the organ represented, the best transverse section indicated by the arrow. S 42, × 40,000.

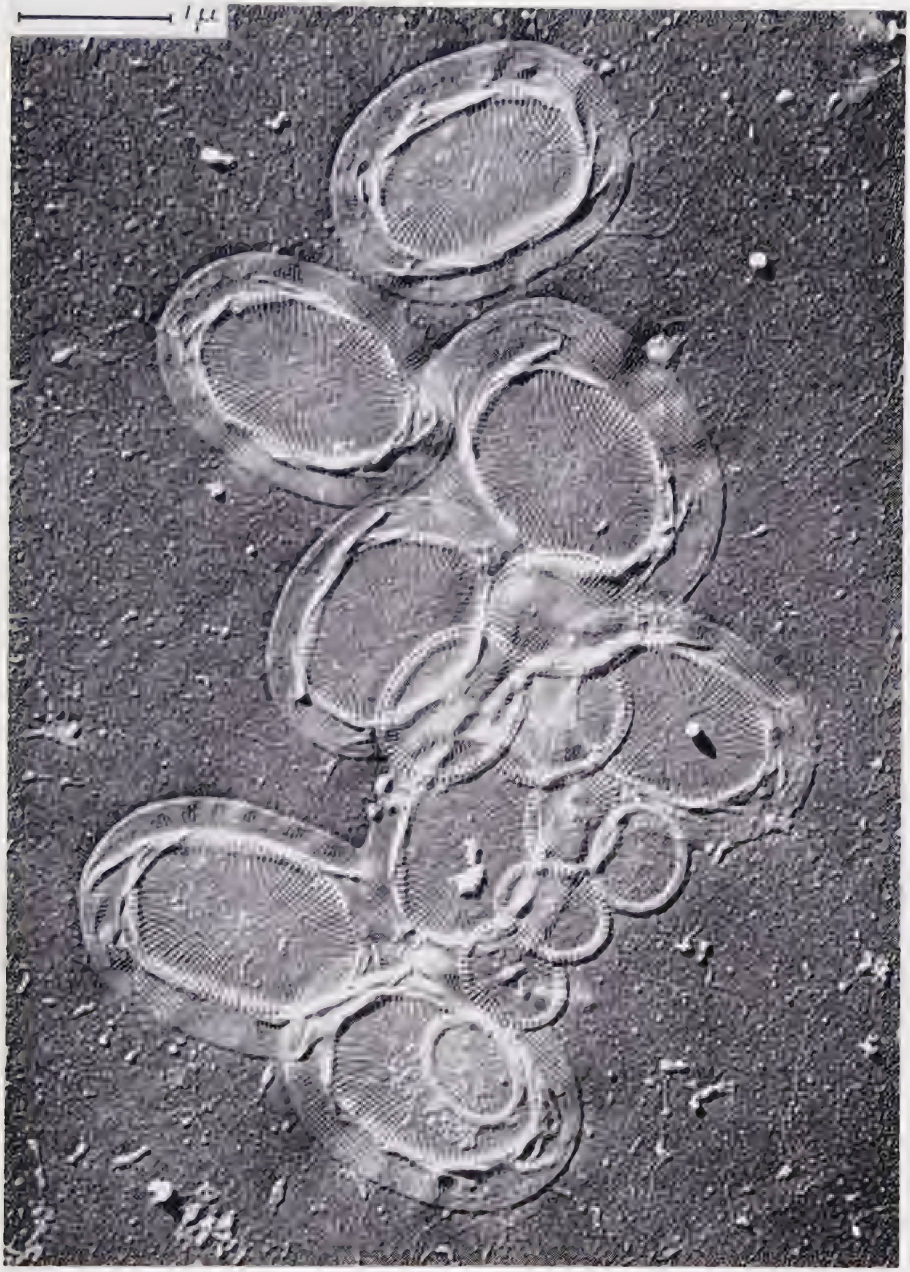
Fig. 20. Another haptonema showing parts of five sections through the axis, the best transverse section marked by an arrow. R 86e, × 40,000.

Fig. 21. Transverse section of a flagellum (right) and parts of three sections of a haptonema (left), the best transverse section in the top left-hand corner (*b*); another view of this from an adjacent section in *a*. S 74 and S 83, × 50,000.

Fig. 22. Transverse section of another haptonema showing advanced 'blistering'; the flexed axis within the distended outer sheath has been traversed three times and in two of these the seven central strands have become detached from the innermost membrane, which is also greatly distended. S 115, × 70,000.

VI

Figs. 23–25. Three successive sections to show details of attachment of two pyrenoids to neighbouring chromatophores; a third pyrenoid, part of the nucleus near the surface, a mitochondrion and other inclusions also visible; for further details see Pl. VII. Mid 502.11, S 97 and S 108, × 12,000.



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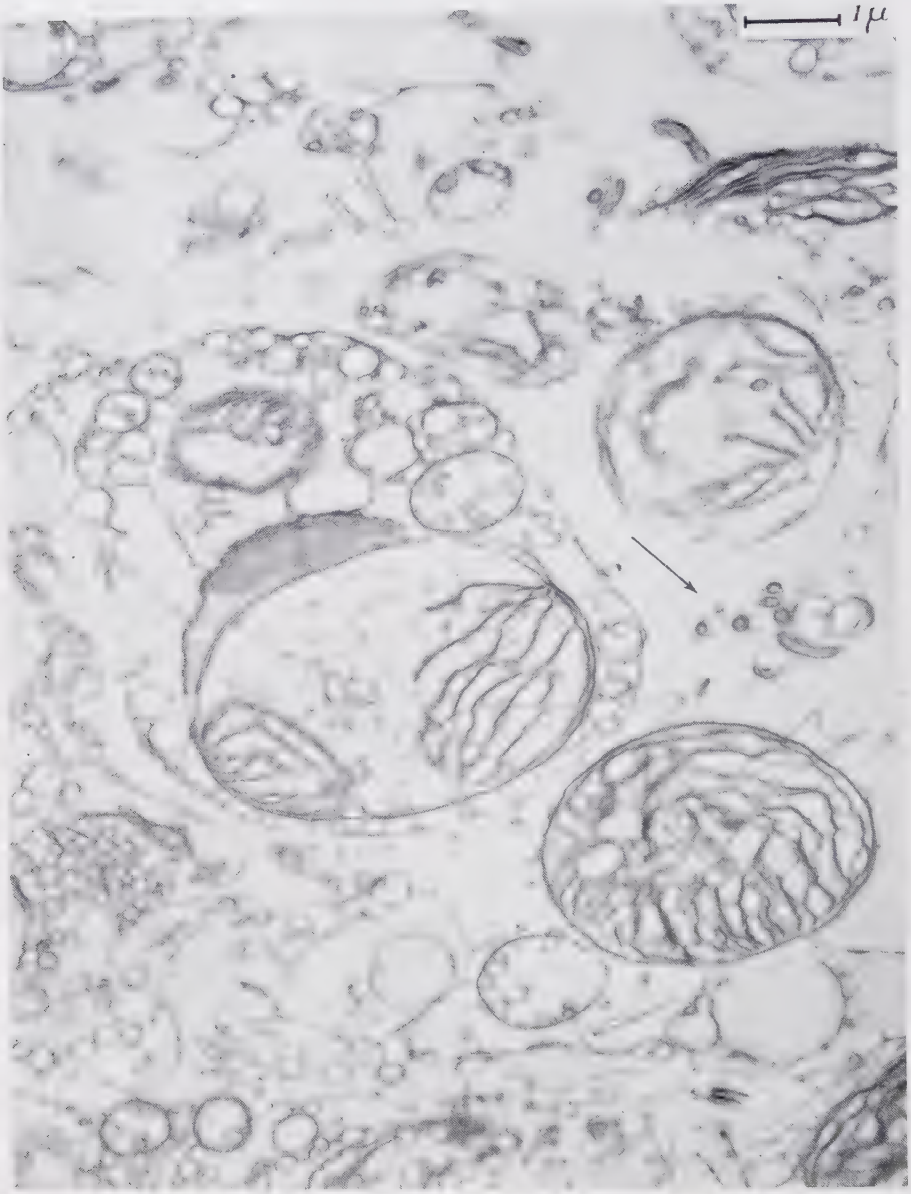

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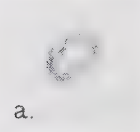

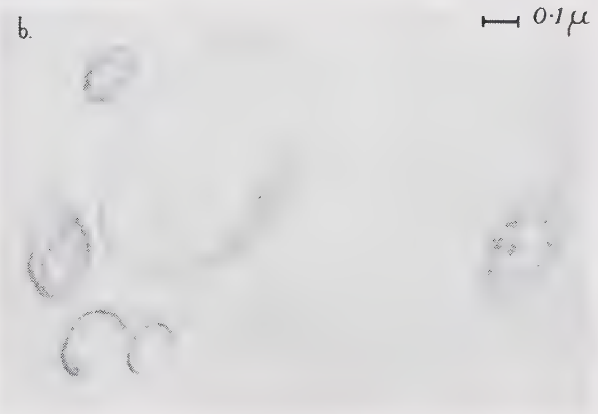

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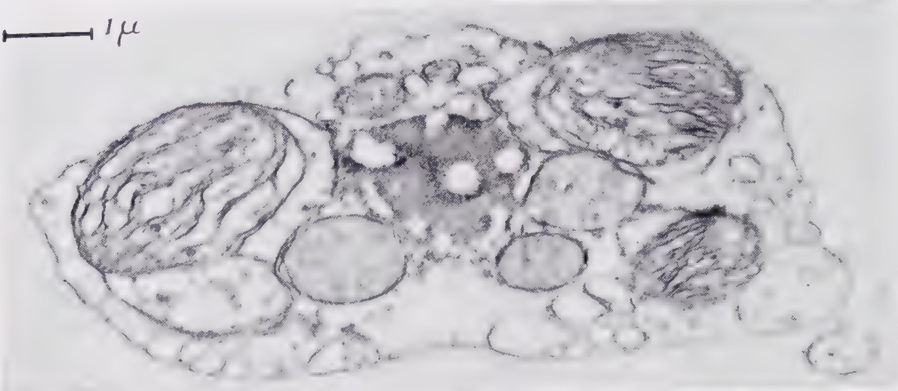

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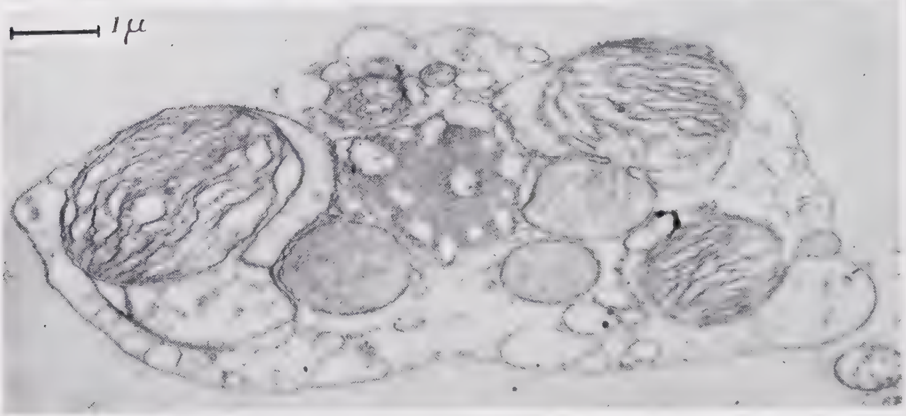

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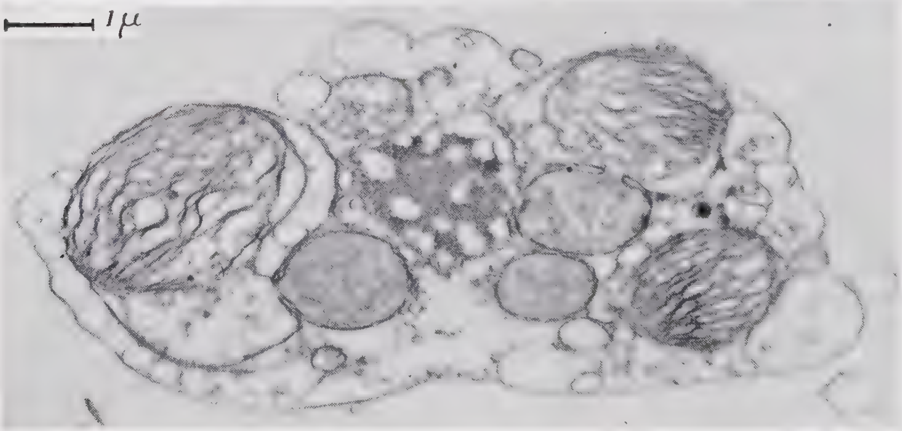

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show distension of the remaining membranes also, and the fibres have become detached and are very clearly countable.

The longitudinal path of the internal haptonema fibres is visible in parts of Figs. 19 and 20. The strands run roughly parallel to each other, though Fig. 19 suggests a slightly spiral path. Minor undulations, never encountered in the peripheral fibres of flagella, are also detectable on individual fibres. There is therefore little doubt that the fibres themselves are different in character as well as in arrangement from those of the flagella.

Since not only the membranes but also the walls of the haptonema fibres are relatively thick, it is probable that both will reveal further microstructure when still higher resolution can be applied. This has not yet been seriously attempted. We likewise lack all anatomical information on the structure of the two extremities of the organ.

With regard to the details of attachment of the appendages to the cell we have some incidental observations for the flagella only. Two examples are illustrated in Figs. 33 and 34, Pl. IX. Fig. 33 is important for demonstrating the spatial relation of the flagellar bases to a group of characteristic vesicles immediately below them which will be described below under golgi material. Fig. 34 is chosen as the best example so far obtained of the structural details of two bases, one in transverse section and the other in longitudinal section, both of which are probably flagella and not haptonemata. If this identification is correct the flagellum seen in longitudinal section seems to lack several of the normal structural details of a basal body, since there is no transverse partition at the level of the cell surface and the interior, which in other cases is hollow, seems occupied here by amorphous dark contents. The transverse section visible (see arrow) beside the posterior end of the longitudinal section shows a stellate outline corresponding to the nine peripheral strands and the centre is similarly filled with dark material. It therefore seems probable that some new features may be expected to exist in the basal parts of the flagella in this organism when they can be further studied.

CONTENTS OF THE BODY AS SEEN WITH THE LIGHT MICROSCOPE

With the light microscope an extremely thin pectic layer covering the body can be distinguished after staining with Ruthenian Red. The principal objects detectable inside the cell are as follows. (i) There are usually two or four golden brown chromatophores, each with internal striations clearly visible from the outer face and probably representing lamellae, and with a variable number (1 to very numerous) of distinct vesicles (0·2–0·5 μ diameter) between the striations, which are soluble in alcohol and which stain intensely with Sudan black (Fig. 4) or Sudan IV especially when the latter is used as a vital stain, these reactions indicating saturated lipoid (cf. Doflein, 1922, for

Ochromonas). (ii) Against the inner face of each chromatophore, and probably attached to it, is a body of variable size (0·5–1·0 μ), usually non-refractile and not staining with Sudan IV, which is probably a pyrenoid (cf. Schwarz, 1932; Chadefaud & Feldmann, 1949; Magne, 1952). A pink coloration of the surface with Sudan IV suggests the possible presence of a lipoid envelope covering this body. (iii) The cell nucleus commonly lies between the chromatophores but is liable to be displaced towards the cell surface from time to time. (iv) Two other bodies (0·5–1·0 μ), one spherical and one rod-shaped, can sometimes be seen close to the nucleus. (v) One or more food vacuoles are often present which move about the body from the non-flagellar pole towards the region of the pyrenoids before their contents are digested. (vi) Usually two or three leucosin vesicles of small size, but sometimes as large as 3 μ, are confined to the non-flagellar half of the cell and stain rose-violet with cresyl blue, a reagent which also picks out (pale blue) the very small muciferous bodies scattered over the cell surface but especially numerous near the flagellar pole, and some tiny bright blue granules perhaps associated with them. (vii) Osmium tetroxide stains one or two bodies of unsaturated fat

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Explanation of Plates VII–X

VII

Fig. 26. A further section from the series in Pl. VI, adjacent to Fig. 25. Mid 501.14, × 12,000.

Fig. 27. Enlarged detail from Fig. 26 to show the pyrenoid (*py*) and neighbouring structures more clearly, including part of nucleus (*N*) and a mitochondrion (*m*). Mid 501.15, × 20,000.

VIII

Fig. 28. Section of a cell with an undisturbed covering of scales and also showing the nucleus (*N*) and nucleolus; two obliquely cut flagella in the neighbourhood. S 71, × 26,000.

IX

Fig. 29. Section of a cell with an undisturbed covering of scales and also showing part of a leucosin cavity, muciferous bodies on the surface, two sections of flagella beneath the scales and therefore belonging to the cell, part of the nucleus, two fat bodies, mitochondria and other organelles also visible. S 104, × 16,000.

Fig. 30. Section of two detached scales and a flagellum to show relative sizes and shapes. S 21, × 30,000.

Figs. 31, 32. Two adjacent sections through a group of muciferous bodies. S 88 and S 78, magnification uncertain.

Fig. 33. Part of a section in the region of the flagellar bases. S 197, × 20,000.

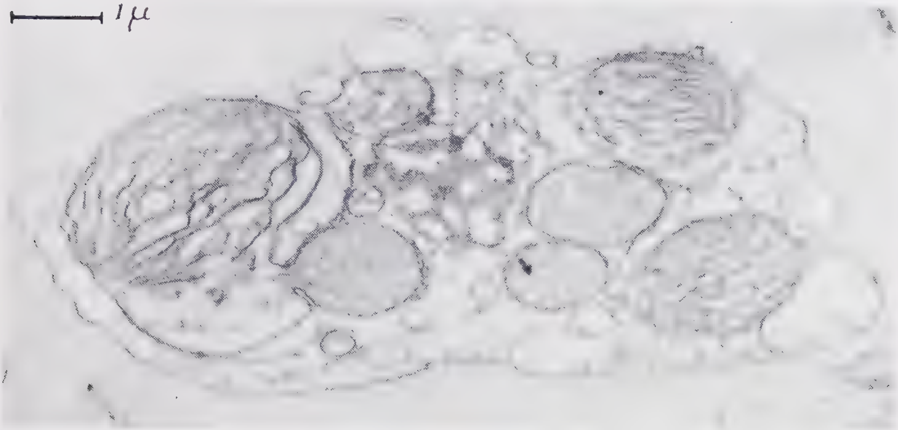
Fig. 34. Part of a section in the region of the flagellar bases, one in longitudinal section and the other in transverse section (arrow); adjacent organelles include leucosin cavity and muciferous bodies. S 202, × 28,000.

X

Fig. 35. A cell showing nucleus (*N*), two chromatophores, a food vacuole, a fat body and golgi vesicles. R 88c, × 10,000.

Fig. 36. A cell showing nucleus, parts of leucosin cavities, mitochondria, three chromatophores and golgi area. S 91, × 10,000.

Fig. 37. Details of the golgi area of Fig. 36 more highly magnified and placed at a different angle on the page; part of the nucleus (*N*) and two chromatophores (*c*) also present. S 92, × 30,000.



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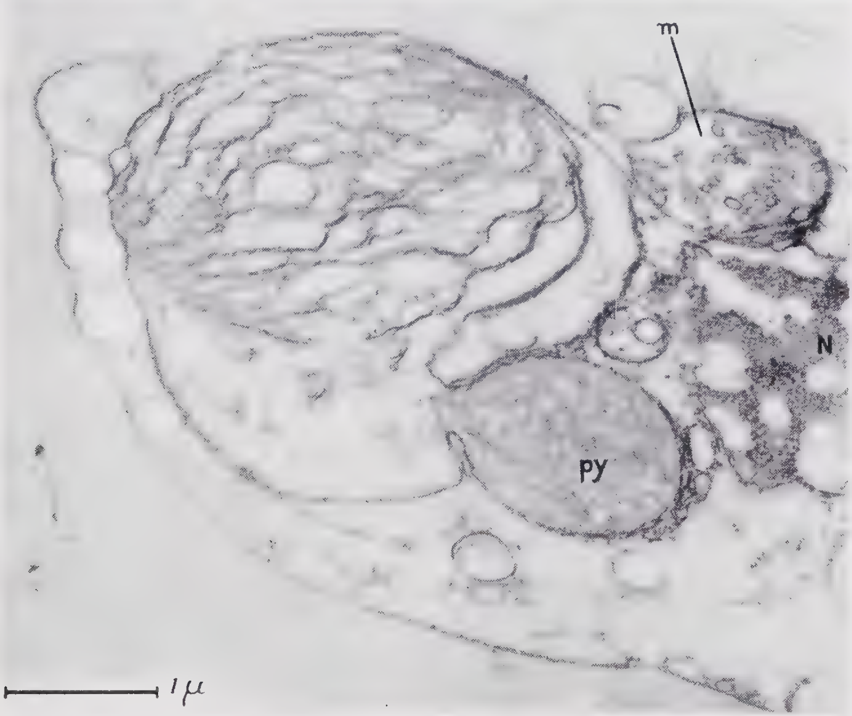

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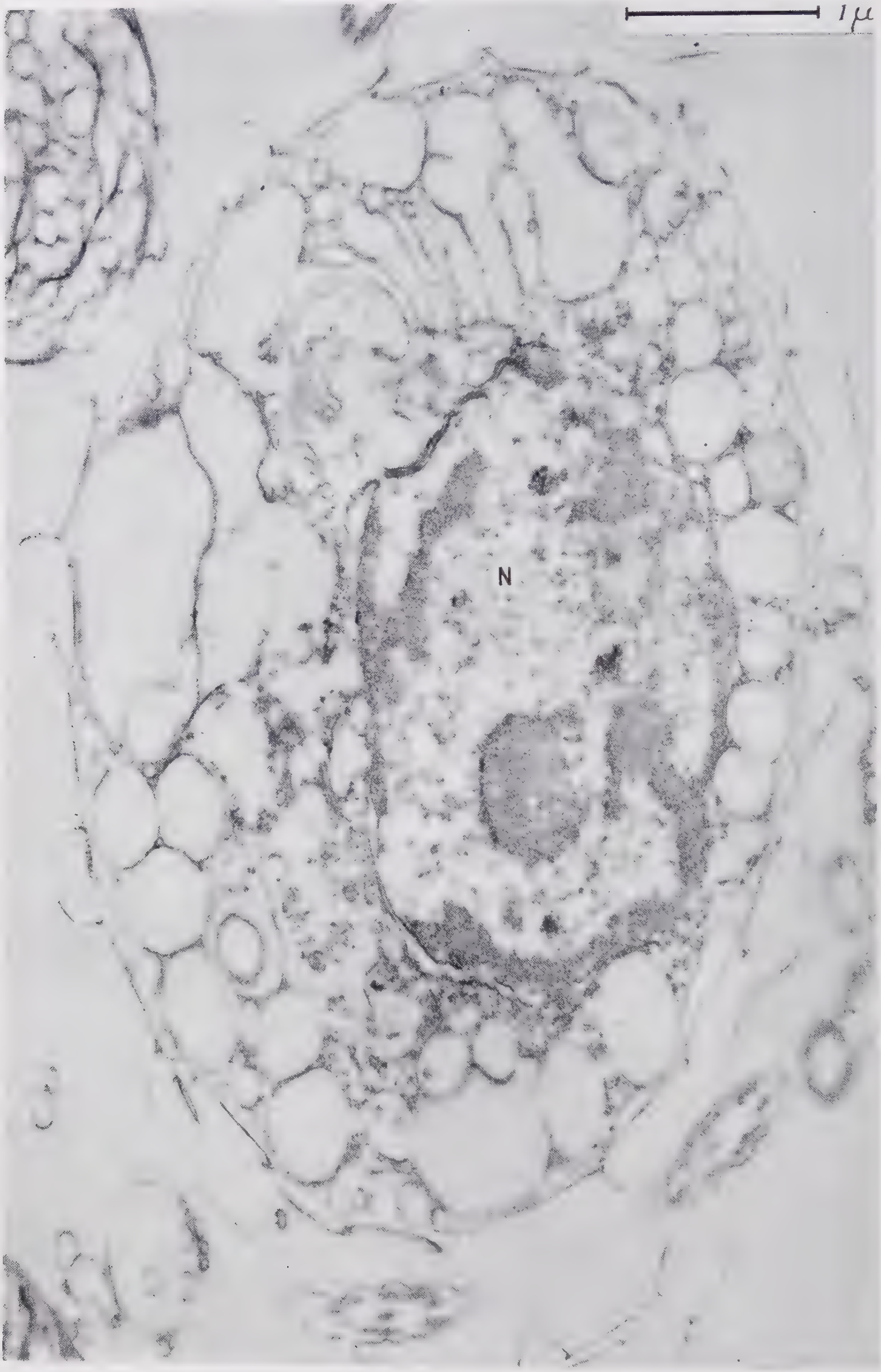

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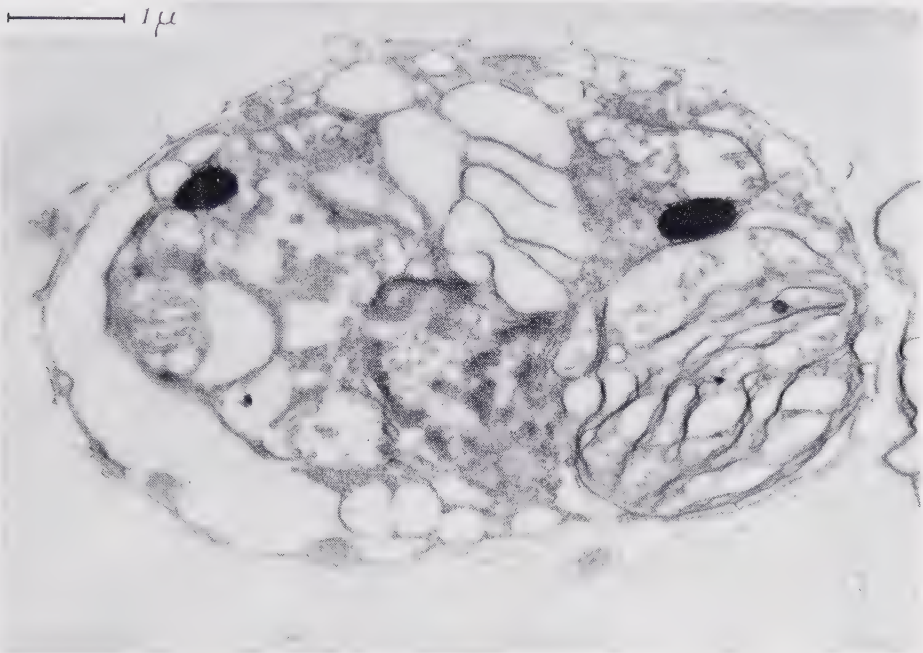

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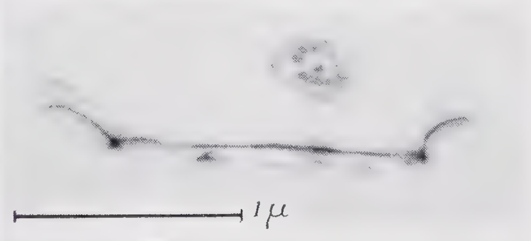

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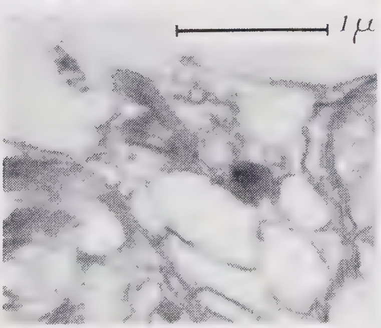

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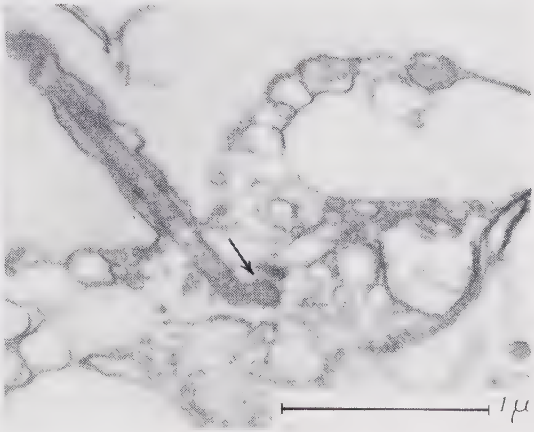

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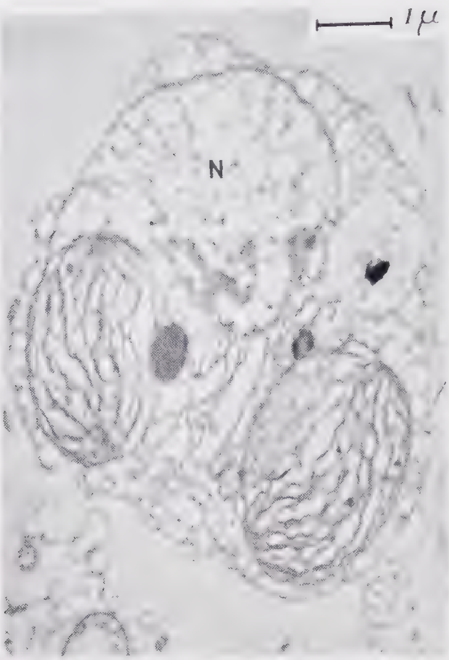

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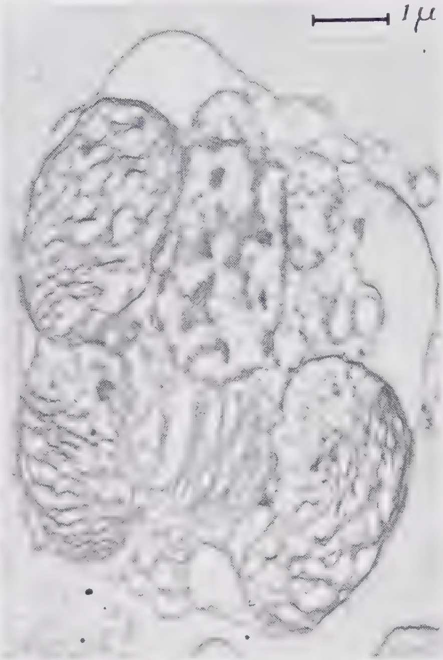

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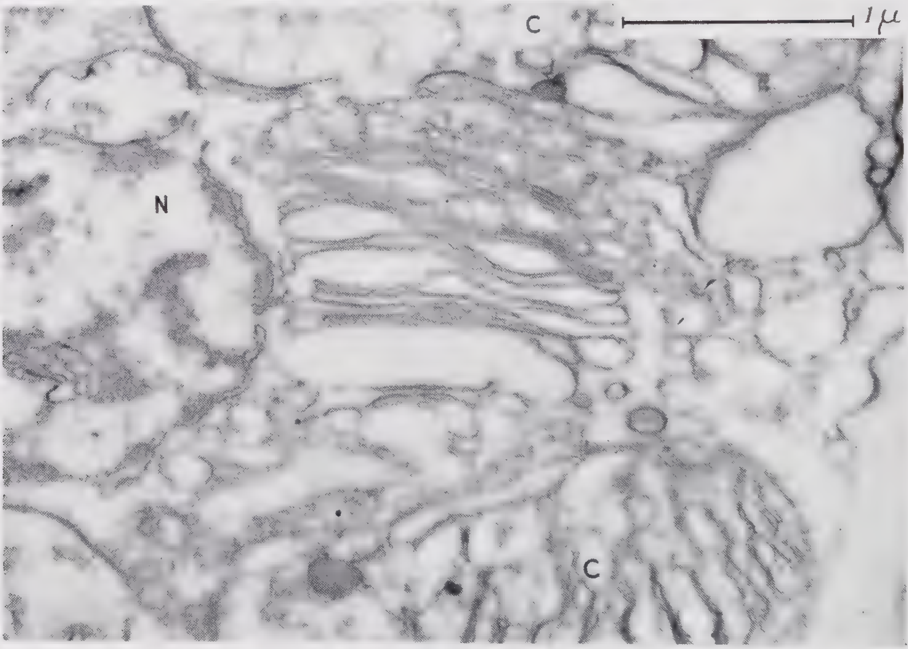

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which occur in the cytoplasm. (viii) A number (of the order of 6–8) of granules of fairly uniform size (0·25 μ) stain with Janus green, which are probably mitochondria. (ix) Janus green also stains faintly one, or more rarely two, much larger bodies of the order of 1 μ in diameter situated near the flagellar pole (see above) and perhaps representing the golgi material identified with the electron microscope (see p. 221).

The positions of these various components in life are indicated in Figs. 1–7 (p. 212), though most of them can change with respect to the flagellar pole in the course of metaboly of the body.

CONTENTS OF THE BODY AS SEEN WITH THE ELECTRON MICROSCOPE

The criteria for recognizing the internal cell organs with the electron microscope are rather different from those available with the light microscope and many are much more precise. Observations on the living cells have, therefore, been of less help in the interpretation of the sections than might have been expected, but, conversely, it is probable that further study with the light microscope after the identity of the various components has been established might amplify knowledge of function and development. With our present interest concentrated on identification, the principal organs detected with the electron microscope will be briefly described.

The Nucleus

The nucleus is the most important organ to recognize at the outset since until this is done certain types of sections of it could easily be mistaken for those of other cell components. There is no such difficulty about a section such as that of Pl. VIII in which the nucleus is the only major organ visible. It contains a single nucleolus, and the rest of the nuclear volume is occupied by irregularly disposed light and dark masses. The darker material is usually aggregated near to the nuclear surface, sometimes very asymmetrically (cf. Fig. 35), and the size of the aggregates varies also, both types of difference being probably associated with different stages in the nuclear division cycle. Other, more confusing, differences are in the overall shape and position, which undoubtedly change with body metaboly. Thus in Fig. 35, Pl. X, the nucleus is very asymmetrical both in shape and position while that of Fig. 36 is of more normal appearance. Parts of tangential sections showing different views of the dense material in an undoubtedly resting nucleus are contained in Pls. VI and VII and in Fig. 29, Pl. IX.

A detail which cannot profitably be discussed at the present stage is that of the nuclear membrane. This is known to have a highly complex structure in all animals and plants in which it has been carefully examined. To study it effectively requires higher magnification than those used here, but attention can be drawn to the part of the nuclear surface included in Fig. 37, Pl. X, and to the nucleus in Pl. VIII to substantiate the statement that in this as in other cells the interior of the nucleus is separated from the body of the cytoplasm not only by membranes but also by a translucent, apparently liquid, layer which, in any one section, appears to be discontinuous but which could equally be a single circum-nuclear vesicle ('cisterna' of Palade & Porter, 1954), or perinuclear space bridged at intervals.

Chromatophores

Larger than the nucleus and conspicuous in most sections are the chromatophores. These are very easily distinguished by their laminated structure, which is comparable to that of many other lower plants. As in *Synura* (Manton, 1955), Phaeophyceae (Leyon & von Wettstein, 1954; Manton, 1957*a*), etc., the laminae are compound, but they do not here fill the entire organ, much of which seems to consist of storage space. The nature of the storage product is unknown and only small traces of it are still present in most sections. Some granular material and a few osmiophilic vesicles are commonly present between the laminae (Fig. 27, Pl. VII), though the lipoid globules which stain so distinctly with Sudan IV on the fresh material are entirely dissolved by the reagents used for embedding and are only traceable as empty vesicles.

The pyrenoid

A very distinctive part of the chromatophore is the pyrenoid. When encountered in isolation a pyrenoid in the sense used here is a highly characteristic spherical body, 0·5–1 μ in diameter, containing dense granular material and surrounded by a system of compound membranes. In order to detect attachment to a chromatophore a very special plane of section is required, since there is only a narrow neck joining the two organs. However, a fortunate series of sections showing this feature in two separate pyrenoids in one cell is contained in Pls. VI and VII. The pyrenoid is apparently in continuity with the storage part of a chromatophore,[1] though the opacity of the contents is very different. We have no interpretation to offer about the other structural details, detectable especially in Fig. 27, Pl. VII.

Mitochondria

Fig. 27, Pl. VII, also contains a good example of a mitochondrion (labelled *m*). The microanatomy of these organelles is now so familiar that little need be said except to explain that the cylindrical villi which occupy the lumen in place of the cristae of many animal cells differ in no essential way from those of other examples in the lower plants, notably *Synura* (Manton, 1955), the brown algae (Manton & Clarke, 1956; Manton, 1957*a*) and *Vaucheria* (Greenwood, Manton & Clarke, 1957).

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[1] See footnote on p. 224.

Golgi apparatus

A slightly less familiar component is illustrated in Pl. X, that has many points in common with the Golgi apparatus of certain animal cells and of some plants, notably *Scytosiphon* (Manton, 1957), wheat roots (Hodge, Martin & Morton, 1957), and *Sphagnum* (Manton, 1957). Attention has already been directed to the position of a compact group of vesicles immediately below the flagellar bases in Fig. 33, Pl. IX. Their characteristically flattened and compact arrangement as well as their position in relation to the nucleus and chromatophores are better displayed in Figs. 35 and 36, Pl. X. The additional details visible in the more highly magnified micrograph of Fig. 37 are in such striking agreement with those of many published micrographs of golgi in animals that the identification as the same organ here seems virtually unassailable unless on biochemical grounds which have not yet been investigated in this material.

Fat bodies

Unsaturated fat bodies, easily identifiable as such by their opacity following osmic fixation, are of common occurrence. They are variable in size and not very numerous; two are contained in Fig. 29, Pl. IX, and also in Fig. 35, Pl. X.

Leucosin

Leucosin in sections is always represented by empty spaces owing to its ready solubility in the alcohols used for dehydration; such spaces are very liable to mechanical distortion, but a demonstrably undistorted cavity bounded by a membrane and almost certainly of this nature is contained in Fig. 29, Pl. IX, beneath the undisturbed covering of scales; parts of very similar cavities are visible in Fig. 34, Pl. IX, and Fig. 36, Pl. X.

Muciferous bodies

The lenticular vesicles of slightly opaque material visible between the leucosin cavity and the covering of scales in Figs. 29 and 34 are the muciferous bodies. As surmised from their behaviour when seen discharging under the light microscope these are not merely amorphous drops or granules of material but organelles with a definite microanatomy. This has not been completely worked out, but from Figs. 29, 31, 32 and 34, Pl. IX, it is clear that each organelle is effectively a small-walled compartment with semi-opaque contents. There are signs of further subdivision of the contents, notably in the faint white internal contours in the central body of Fig. 31, and there is certainly anatomical complexity about the outer wall which cannot yet be defined, though characteristic views of relatively median sections can be studied in Figs. 31, 32 and 34. The apparently translucent thickening of the outer wall causes the contents of an undischarged organelle to appear somewhat bean-shaped. Further information about the meaning of these structures is greatly to be desired.

The outer membrane

Covering other parts of the cell, beneath the scales, is a delicate outer membrane. This is demonstrable with special clarity in cells which have lost their scales and is visible in most of those illustrated in Pls. VI, VII and X.

Cytoplasmic vesicles

The most conspicuous of the other cell contents are the vast array of vesicles, most of them apparently empty, though doubtless not so in life, which crowd the cytoplasm between the other organs and which are specially conspicuous near the outside of the cell (Pls. VI and VII). They are probably of more than one kind but, though none is as yet chemically identifiable, they resemble at any rate superficially the cytoplasmic vesicles commonly encountered in other cells of the lower plants, cf. *Scytosiphon* (Manton, 1957*a*).

Food vacuoles

Food vacuoles are distinguishable from other cytoplasmic vesicles by their irregular contents, which sometimes include recognizable objects such as ingested scales. One food vacuole with rather indefinite contents is present in Fig. 35, Pl. X.

Cytoplasmic granules

Finally there are cytoplasmic granules. Since extreme metabolic significance has been attributed to certain types of granules (cf. Palade, 1956, etc.), it is important to notice their presence here even though we have no chemical data to add. Granular cytoplasm fills the interstices between vesicles and other cell organs in all parts of the cell. It may be seen specially clearly in Fig. 29, Pl. IX, and Fig. 37, Pl. X.

BEHAVIOUR OF THE LIVING CELL AND REPRODUCTION

Chrysochromulina chiton shows a marked phototactic response, although there is no obvious stigma and so far no cells lacking chromatophores have been observed.

During swimming the haptonema and flagella are directed backwards, the haptonema usually coiled up close to the body (Fig. 1), but sometimes partly or fully extended. Movement can be fairly rapid for short periods, the cells swimming in straight lines rotating slowly with a jerky movement (as if flinging one flagellum round—probably due to excentric insertion on body). The cells are rarely seen moving with flagella and haptonema in front of the body. A characteristic of this species is a gliding movement without rotating, with flagella held behind body and with haptonema coiled or extended (Fig. 4). As swimming or gliding slows down, the flagella lie further apart from each other.

Quite long periods of anchorage are common. When anchored the haptonema can be extended to any degree from almost completely coiled to fully extended (Figs. 3 and 7). It is, however, rarely entirely straight (Fig. 4); when extended backwards during swimming it is commonly curved at the tip and when attached it is most commonly bent or slightly coiled (Figs. 2, 3 and 5–7).

Phagotrophy is of very common occurrence, the cells ingesting organisms up to about $3\,\mu$ in size (Figs. 1–7). The presence of three food vacuoles in one cell at the same time is not uncommon, but one or two is more usual. The method of ingestion is similar to that described for *C. ericina* (1956, p. 397) and the method of discharge of the contents of the muciferous bodies is as described for *C. kappa* (1955, p. 589).

Reproduction of the motile stage of *C. chiton* is similar to that described for *C. kappa* (1955, p. 592), but the lop-sided movement recorded for *C. chiton* is particularly accentuated in incipient fission stages. Double fission stages are more rarely seen in this species than in *C. kappa*. Cultures of *C. chiton* reach a density of $1\frac{1}{2}$–2 million cells/ml. at the peak of growth. Non-motile stages similar to those already described for the other species (1955, 1956) are then produced, but in this species the scale covering of the motile cell appears to remain fairly intact except for a pore through which the contents have come out and masses of these empty 'skins', clearly visible under the light microscope, can be picked up from the bottom of a flask. The large amoeboid and walled cells, both with finely lobed chromatophores, measure from 7×12 to $9 \times 13\,\mu$, while the daughter-cells with smooth thin walls and fairly finely lobed chromatophores measure from $2{\cdot}5 \times 3$ to $3{\cdot}5 \times 5\,\mu$.

DISCUSSION

Most of the more obvious comparisons with other organisms have already been made in passing, such comparisons being necessarily limited at present by the relative scarcity of plant cell types which have been effectively studied by these methods.[1] The most fruitful external comparison at the present stage probably concerns the pyrenoid. This problematical body, or something closely resembling it, has been recognized by light-microscopists in several different groups of non-starch producing organisms (for literature see Fritsch, 1935; Chadefaud, 1936; Chadefaud & Feldmann, 1949; Magne, 1954; etc.), always as a body external to the chromatophores though possibly connected to them, and characterized by negative staining reactions when tested by most of the ordinary reagents giving diagnostic colouring. Demonstration of organic attachment to an adjacent chromatophore by a long slender neck has been claimed for *Ochrosphaera neapolitana* (Chrysophyceae) by Schwarz (1932) and Magne (1952). The clarity with which the electron microscope has now revealed, in *Chrysochromulina chiton*, the attachment of the pyrenoids to adjacent chromatophores by a short constricted neck makes this one of the most definite facts which has so far been ascertained about such pyrenoids. The probability is thus greatly increased that they are in fact storage organs concerned with some product of photosynthesis, which the original choice of the name pyrenoid necessarily implies. A resemblance to the 'plastid diverticulum' recently described (Manton, 1957*a*) in the zoospores of the brown alga *Scytosiphon* is also perhaps of importance to note.[1]

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[1] A paper (Fauré-Frémiet & Rouiller, 1957) which is closely relevant to the present inquiry has reached us too late to be discussed in the text. The authors have investigated a species of *Chromulina* by means of thin sections with the electron microscope. *Chromulina* differs characteristically from *Chrysochromulina* in the external appendages and in the presence of an eyespot but shows many points of resemblance in the other body organs.

Our ignorance of the chemical nature of the contents of pyrenoids only emphasizes what is in fact the most serious gap in our total knowledge of these organisms, and one which requires methods quite other than straight microscopy, namely the general problem of the gross cytochemistry of the cell as a whole. Except for the leucosin, practically nothing is known about the chemical nature of any of the storage products which appear in abundance in various parts of the cell. If this gap could be filled progress in other matters would undoubtedly follow.

An outstanding problem which is both chemical and developmental concerns the structure and mode of origin of the scales, which at present is difficult even to imagine. That there is a delicate cytoplasmic membrane underlying them is a fact which in itself is less explanatory than might at first be thought. The observations on the membrane have necessarily to be made with fixed material, yet we know that in life the cell can engulf solid food through its surface in spite of the covering of scales, etc. The physical nature of the cell surface cannot, therefore, be fully deduced from the anatomical evidence, and we are equally ignorant of the way in which it may perhaps change with time. There are no data, for example, to indicate whether the scales are produced cyclically following a related change in the cell surface prior to their production, or whether they are in fact being produced all the time as a normal process of growth from the surface as we commonly see it. Several details about the shape and arrangement of the scales are difficult to reconcile with the latter view, though anatomy alone is an insufficient guide in such a question.

Purely anatomical information can, however, still add much to our knowledge of the haptonema, though this must be ascertained comparatively through a range of species before final generalizations can be made. We have shown in *C. chiton* that the anatomical differences from a flagellum are at least as great as had been surmised from their very different behaviour and appearance. We do not, however, yet know the extent of uniformity or diversity within the genus, and in exploring this it is reasonable to hope that our present ignorance about the structure of the extremities, about the mode of attachment to the cell, and, more importantly, about the mechanical principles involved in its movements may also, with further work, be dispelled.

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[1] Since this was written further information on pyrenoid structure in the Chrysophyceae is available from two sources. Hovasse & Joyon (1957) describe pyrenoids immersed in the structure of the chromatophore in a species of *Hydrurus*, and we have found a similar condition in some undescribed species of *Chrysochromulina*. The special condition of *C. chiton* is therefore not universal in the group.

FORMAL TAXONOMIC DIAGNOSIS

Chrysochromulina chiton sp.nov. Parke & Manton

Motile cells showing some metaboly, sphaeroidal to ovoid with a flattened flagellar pole depressed centrally across one axis, 5–8 (exceptionally 4–10) μ in diameter. Two flagella and one haptonema arising close together slightly off-centre from the depression; the flagella subequal, or of equal length, homodynamic, $2\frac{1}{2}$–$3\frac{1}{2}$ times body length, smooth, apex gradually attenuated to a small knob (E. M. observation); the haptonema, thinner than the flagella, 4–5 times body length when fully extended, with a club-shaped tip and an internal structure of three concentric membranes surrounding a ring of seven 'fibres'. The very thin periplast of a pectic nature covered by very thin, transparent, oval, two-layered, sculptured, exceptionally large, dimorphic scales, visible when dry under the light microscope, details visible only under the electron microscope. Large scales oval, bipartite, saucer-shaped, with a wide rim delimited from an oval centre by a ridge, $1{\cdot}9 \times 2{\cdot}4$ to $2{\cdot}2 \times 2{\cdot}9\,\mu$, with the base showing a radiating pattern of ridges on the surface towards the body, the outer surface patternless. The small scales, round to oval, $0{\cdot}7 \times 0{\cdot}9$ to $1{\cdot}1 \times 1{\cdot}4\,\mu$, with the relatively patternless rimmed face away from the body and the face with the pattern of radiating ridges towards the body. Scale distribution a single layer over the body with small scales filling interstices between large.

Cells uninucleate, no stigma. Chromatophores appearing striated, usually two or four, occasionally one or six, golden brown; in motile phase usually ellipsoid or oblong, parietal, with a single globular body (pyrenoid) attached by a constricted neck to inner face near one margin slightly nearer to the non-flagellar pole than the flagellar pole; in non-motile phase deeply lobed or stellate. Lipoids and leucosin produced. Ejectile muciferous bodies generally distributed in peripheral cytoplasm but usually more numerous at flagellar pole. Nutrition phototrophic and/or phagotrophic. Non-toxic to fish.

In motile phase asexual reproduction by fission into two, three or occasionally four daughter-cells of equal or unequal size; in non-motile phase reproduction (asexual?) by successive fission of amoeboid cells to produce four daughter-cells with thin smooth walls; motile phase probably liberated from walled daughter-cells through a pore.

Isolated by Miss Dorothy Ballantine from sea-water samples collected by her during cruise, May 1955.

Habitat. The sea at position lat. N. 47° 36′, long. W. 04° 18′ (23 May 1955, Plymouth no. 146, type culture) at 50 m; at position lat. N. 47° 44′, long. W. 07° 11′ (17 May 1955, Plymouth nos. 149, 150) at 5 m; at position lat. N. 50° 13′, long. W. 04° 22′ (29 May 1956) at surface; at position lat. N. 50° 09′, long. W. 04° 16′ (19 June 1957, very fine tow-net sample); at position lat. N. 50° 02′, long. W. 04° 22′ (16 July 1957) at surface; at position lat. N. 50° 11′, long. W. 04° 14′ (24 July 1957) at surface; at position lat. N. 50° 02′, long. W. 04° 22′ (17 Sept. 1957) at 5 m, 10 m, 20 m and 50 m, and at position lat. N. 50° 11′, long. W. 04° 14′ (15 Oct. 1957) at surface. Type culture deposited with the Culture Collection of Algae and Protozoa, Cambridge.

Cellula motilis aliquanto formam mutans, sphaeralis vel ovalis; extremitate, qua inserta sunt flagella, centraliter depressa trans alterem axem; lata 5–8 μ (rare 4–10 μ); duobus flagellis sed et uno haptonemate conjunctim exorientibus paululum remotis depressione centrali; flagellis subaequis vel aequis, homodynamicis, longioribus 2½–3½plo quam cellula, teretibus, apicibus paulatim attenuatis sed terminatis minutis bulbis (ut videtur per microscopiam electronicam); haptonemate teneriore quam flagellis, longiore 4–5 cellula maxime extenso, extremitate claviformi; haptonemate induto 3 membranis concentricis, 7 filamentibus axialibus dispositis in annula ut videtur in sectionibus transversis; periplasto tenuissimo, pectico natura, induto tenuissimis, diaphanis, ovalibus, sculptis squamis, grandibus praeter modum, duobus stratis compositis et sub duabus formas existentibus, visibilibus siccis per microscopiam photicam, minuta structura tamen obscura nisi per microscopiam electronicam; squamis majoribus ovalibus, bipartitis, crateriformibus, longis 2·4–2·9 μ, latis 1·9–2·2 μ, lata margine circumscripta ab ovali areola centrali per angustum jugum, cuius areolae aspecto interno, contra cellulae corpus, sculpto radiantibus rugulis, aspecto externo sine rugulis; squamis minoribus circularibus vel ovalibus, longis 0·9–1·4 μ, latis 0·7–1·1 μ, aspecto interno cum radiantibus rugulis, aspecto externo cum jugo circumscribente, sed rugulis relative defectis; squamis distributis unico strato per superficiem cellulae, minoribus replentibus interstices inter majoribus. Nucleo unico, stigmate defecto; chromatophoris apparente striatis, generaliter 2 vel 4 (rare 1 vel 6), aureobrunneis; in cellulis ad statum motilem chromatophoris generaliter ellipsoidalibus vel oblongis, parietalibus, unico corpore pyrenoidali globulari affixo per isthmum angustum cuique chromatophoro, prope marginem, paululum propiore extremitatem cellulae qua inserta sunt flagella; in cellulis ad statum immotilem chromatophoris profunde lobatis vel stellatis; cellula materias lipoidales et leucosinum producens; ejectilibus corporibus muciferis diffuse distributis in cytoplasmate superficiali, generaliter tamen abundantioribus ad extremitatem cellulae qua inserta sunt flagella. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Cellula ad statum motilem generans per fissionem ad 2, 3 vel rare 4 cellulas filiolas producendas, magnitudine paribus vel disparibus; cellula ad statum immotilem generans (? asexuale) per fissiones sequentes cellularum amoeboidalium ad 4 cellulas filiolas producendas cum parietibus tenuibus et teretibus; ex quibus cellulis filiolis, cellula ad statum motilem per porum liberata (quod maxime potest).

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SUMMARY

The description of a new species of the genus *Chrysochromulina*, *C. chiton*, includes for the first time some evidence from the electron microscopy of sections in addition to the techniques previously used. Important diagnostic characters are the exceptionally large scales, which are shown to be of two kinds, the larger being saucer-shaped, and the smaller plate-like with a narrow rim flexed towards the upper surface; the structure and arrangement of the two types of scale on the body are described. Anatomical facts are given for the first time for the internal structure of the haptonema; in this species this organ consists of three concentric membranes surrounding a ring of seven fibres or tubes, the centre of the haptonema being hollow; this combination of characters distinguishes the organ fundamentally from a flagellum. Micro-anatomical facts are also given for the following major cell organs: the nucleus, the chromatophores including the pyrenoids, mitochondria, putative golgi material, muciferous bodies, the flagellar bases (preliminary observations only). Other distinguishable cytoplasmic components include the surface membrane, unsaturated fat bodies, vesicles of various kinds and granular protoplasm in the interstices.

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THE BEHAVIOUR OF *LITTORINA LITTOREA* (L.) UNDER NATURAL CONDITIONS AND ITS RELATION TO POSITION ON THE SHORE

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(Text-figs. 1 and 2)

The four common species of British littorinids are all browsers (mainly on algae), and each characteristically occupies a wide but fairly well-defined zone relative to the tide marks. *Littorina littorea* is a normal and often common member of the gastropod population of the middle shore wherever rock or pebbles are found, and also occurs in numbers on wooden structures such as piers and groynes. More rarely is it found on sandy or muddy shores, particularly if they are sheltered and poorly drained. Only occasionally does it become established above H.W.N., whilst it decreases in numbers below E.L.W.S., even where there is good holding ground. This much is well known, but reasons have been given (Smith & Newell, 1955) for believing that on the shore at Whitstable individual winkles tend to remain faithful to the particular beach level which they adopted during the first year of life after larval settlement.

In contrast to truly sedentary animals, such as barnacles and most lamellibranchs, winkles move about, and were their movements completely at random this constant pattern of zonation would in time be upset. (This must apply not only to littorinids, but to all intertidal animals with broadly similar habits.) An inspection of any shore at low water gives the impression after the tide has receded that the vast majority of animals are settled in situations which best protect them from desiccation, extremes of temperature, insolation and other adversities, but predators and browsers must leave such positions in order to obtain food. Yet, with a few notable exceptions the feeding excursions have not been studied in detail. The present investigation was designed to show how winkles maintain their pattern of zonation, and was done partly on the shore at Whitstable and partly in the laboratory at Queen Mary College.

THE DISTRIBUTION OF WINKLES ON THE SHORE AT WHITSTABLE

The distribution of the winkle population shows no general relation to gravity, light, tidal level or beach slope. Nor does desiccation, itself correlated with time of exposure and tidal level, seem obviously to affect the density of the population. Some winkles are found on top of stones, others at the sides of stones and others again on the vertical surfaces of groynes. It is true that those winkles on vertical surfaces always come to rest with their anterior ends uppermost, but on stones an apparently random orientation prevails.

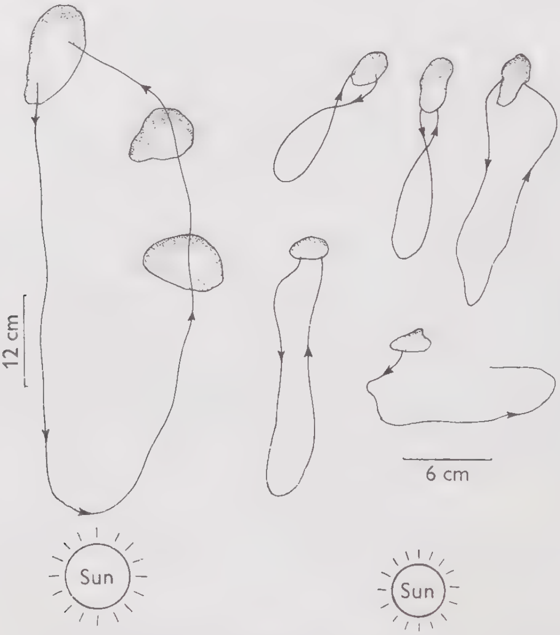

Fig. 1. Selection of tracks made by winkles crawling in the sand near mid-tide level in bright sunlight. September 1956.

Although winkles seem to be distributed in an apparently random way if broadly viewed, they can be noticed to aggregate and form clusters, especially at the junction of two surfaces as, for example, where planking joins a post on a groyne, and also in pools, where winkles are obviously more densely congregated than in drier situations.

On certain parts of the beach, patches of fine sand are interspersed with pebbles and these serve to record the tracks made by winkles when crawling. Such tracks are roughly U-shaped and can often be seen to radiate out from and back to stones on which the animals have settled.

On the vertical surfaces, such as the planking of groynes, winkle tracks are less obvious but, under favourable conditions, they can be seen to take the form of a downward, horizontal and upward limb. In this they resemble the U-shaped tracks in the sand.

During the summer months, when winkles are most active, the tracks in the sand give no clear indication of uniform orientation. Indeed, they appear to be completely random, but, as will be shown later, this appearance is misleading and the apparent randomness of their direction is attributable to the difficulty of following by eye alone a maze of criss-crossing tracks. Yet the very fact that all tracks pursue a fairly straight course for some considerable distance is, in itself, suggestive of orientated responses. The vast majority (roughly 95%) of these tracks consist of an outward and a homeward limb and some that appear to consist of a single limb only may be of the same general form, one limb having been obliterated by wavelets if crawling had begun before the tide had left the zone completely. Examples of some of these tracks are shown in Fig. 1. A few winkles pursue a fairly steady course and show no reversal of direction, but the reason for this is not known.

When crawling on stones winkles can be observed to feed by rasping off organic surface deposits such as diatoms and small algae. They will also browse on *Ulva* and *Enteromorpha*. Indeed, certain areas of groynes are kept clear of *Enteromorpha* by this means, and it must be supposed that any winkle quickly exhausts the food supply in the immediate vicinity of the place on which it has settled.

THE MAINTENANCE OF ZONATIONAL POSITION

Gowanloch & Hayes (1926) presented evidence, from observations on marked specimens, that winkles migrate back to the particular level on which they normally dwelt, after displacement to other situations, whilst Moore (1936), who marked winkles with cellulose paint with a view to following the growth rates of particular specimens, was able to re-collect many of these quite considerable periods after their replacement in roughly the same area. Many of Gowanloch & Hayes's specimens got lost and their results are somewhat equivocal, so that further observations are desirable, particularly since they state that winkles seem to move a few metres at each tide—a view which conflicts with observations recorded in the present paper.

The following observations are typical of several which have been made on the shore at Whitstable, and they show that, if left undisturbed, winkles will return to similar but not necessarily identical positions, and that if removed from the place where they have settled, their chances of getting back home are reduced.

Ten winkles, clustered around a hexagonal nut joining the planking to a wooden groyne post at about the level of H.W.N., were each marked with a dab of yellow enamel before the tide reached them at 13.15 h on 25 September 1956. Twenty-four hours later, i.e. after two tides, five winkles were in the same position, two were within 1 ft. on the planking whilst three could not be found. On 27 October, 1 month later, five were still within 4 yd. of the nut and of these, one was still on the nut itself. On 28 December 1956, one winkle was still on the nut, three were within 5 yd. on the planking whilst the rest could not be found. In short, these observations show that winkles remain restricted to a particular situation, and it must be stressed that the situation studied was one in which the winkles were in a most unstable state and were subject to severe wave action—far more severe than if they had been on horizontal surfaces.

Twenty winkles were clustered one above the other along the junction of a vertical post with the planking of a groyne. Of these, ten were left undisturbed and were marked with a spot of blue paint, whilst ten were lifted and marked with a spot of yellow as well as blue paint and then placed in the muddy sand 2 ft. away from the base of the groyne. After 24 h, i.e. two tides later, of the undisturbed winkles three were in exactly the same place, two were in a similar situation but on the next post, two were at the base of the groyne whilst three could not be found. Of those which had been removed by 2 ft. from their place of settlement, two were in the same place, two in a corresponding position on the next post and six could not be found. After 4 weeks only five winkles marked with a spot of blue paint could be found, but all were within 5 yd. of their original position. None with yellow and blue spots were found. This shows that displacement from the site of settlement tends to decrease the chances of a winkle getting back to its original position.

It must be realized that under natural conditions winkles are continually liable to displacement by wave-action, but that this is usually limited to the time at which waves are breaking over the zone on which they have settled, and once they have been covered by the advancing tide wave-action is too slight to dislodge them except on very steep parts of the shore. Winkles settled on very dry situations and adhering merely by the adhesive action of a film of dried mucus instead of by the foot are particularly liable to be dislodged, but there is reason to suppose that those animals sticking to stones or groynes by the adhesive action of the foot are not often detached from their place of settlement. This has been confirmed by numerous observations on the shore. It is also clear from considerations of the relation of the efficiency of wave-action relative to beach-face slope (Newell, 1954) that high-level winkles are more liable to displacement by waves than those on the flatter slopes below H.W.N. Yet the previous results (Smith & Newell, 1955) show that the exchange of winkles between beach levels is strictly limited, whilst observations on marked winkles moved to different situations gave no evidence that the animals return to the particular level from which they were collected.

Before the tide has ebbed to its finality, practically all winkles are at rest.

Those on groynes or on dry stones settle down almost as soon as the water leaves them, but those in pools crawl about for periods of up to about 3 h after the tide has receded. But eventually even those left in situations where they are permanently covered by water become quiescent, as is shown by an observation of 14 September (a time of the year when winkles are still very active), when out of forty-five winkles in a shallow pool near mid-tide level, forty-three had settled down $5\frac{1}{2}$ h after full tide. Yet, as soon as the flood tide reaches them, all the winkles begin active crawling once again.

These observations suggest that winkles remain stationary during the greater part of each tide even when they are submerged, whereas Haseman (1911) believed that they crawled continuously 'at random' when covered by water and that their activities ceased only when they were out of water on dry surfaces, a view which is implicit in the writings of others, for example Kanda (1916) and Barkman (1955). It would, of course, be most interesting to follow the movements of individual winkles for some time after the tide had covered them, but this is practically impossible in the turbid water at Whitstable. Nevertheless, results, which are probably comparable to those which would have been obtained under natural conditions, can be arrived at by a study of winkles in a tank with a fluctuating water level.

ADHESION

Winkles on vertical or steeply inclined surfaces almost invariably come to rest with their anterior ends uppermost. After some time of exposure many of them secrete a film of mucus (as noted by Wilson, 1929) around the rim of the shell and this hardens sufficiently to support the animal's weight. Then the foot is withdrawn and the operculum closed so that the animal is in no danger of rapid desiccation. Wilson noted that a mere puff of wind will topple winkles from the rocks to which they are stuck by mucus, and further states that those which come to rest with the head downwards always topple off when the foot retracts. I have been unable to confirm this last observation of Wilson's. Indeed, on the contrary, as can easily be demonstrated, winkles are far more unstable on sloping surfaces when orientated head-uppermost than when the head is downwards. This is at first sight somewhat surprising, but this very instability has survival value in that, should any winkle come to rest above the high water mark, the slightest disturbance will cause it to roll down to a level which will be reached by the next tide and so avoid the possibility of not being wetted, possibly, until a fortnight later.

CRAWLING AND FEEDING

When a winkle is about to begin to crawl, the foot and head are protruded through the shell aperture and the operculum on the metapodial region is turned upwards and backwards above the mesopodial region so that the creeping sole of the foot can be applied to the substratum. When crawling on either a vertical or a horizontal surface winkles continually 'test' it with their tentacles, which are extended obliquely outwards from the head, but not at any constant angle for they are in almost continual movement, first the right and then the left feeling the substratum. At first sight it would seem that these bending movements of the tentacles, on which it will be remembered the eyes are borne, would preclude the possibility of the animals maintaining any constant orientation to light, but observations made it clear that only that part of the tentacle distal to the eye is involved in the bending and so the eyes maintain a steady position during crawling (Fig. 2). The degree of extension of the head is such that the eyes, placed about one-third of the way up the tentacles, are a short way in front of the pigmented edge of the mantle, which, with the shell, prevents light from the rear from entering them.

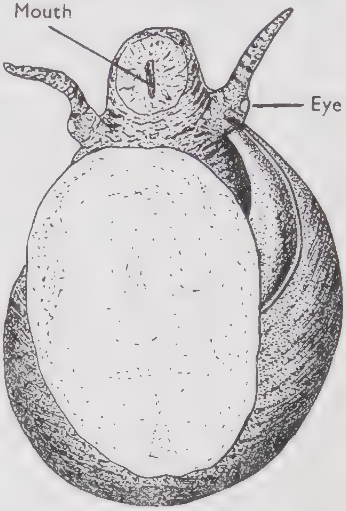

Fig. 2. Winkle crawling on a glass plate—viewed from below.

It can also be noticed that when crawling the animal makes feeding movements, the radula being alternatively protruded and retracted about once every second.

The rate of crawling on wet sand, as determined by an average of twelve specimens on 14 September 1956, was 2 cm/min, which is in very good agreement with the figures of Gowanloch & Hayes (1926) who give an average rate of crawl on rock surfaces as 1·3 m/h which equals 2·1 cm/min. It was found, however, that in the laboratory, either on vertical or horizontal glass plates, winkles can attain a rate of crawl of 10 cm/min. The rate of crawling seems, in part, to depend on the temperature (see p. 236).

In bright light active winkles always respond to a sudden decrease in light intensity by a rapid but momentary withdrawal into the shell. That is, they respond in the same manner as to any other sudden change in the environment, but the withdrawal response to shadows quickly wanes.

FACTORS INFLUENCING THE SEASONAL ACTIVITIES OF WINKLES

So far the account of the behaviour of winkles on the shore at Whitstable has been confined to that seen in the autumn, which is substantially true for the spring and summer months also. But from November onwards, through the winter until March, the winkle population is largely inactive. For example, on 17 November 1956, a dull, calm day, practically no winkle tracks were discernible. On the following day, with the sun showing as a clear disc through the mist, only one winkle was seen to be moving after the tide had receded. The same inactivity on the shore prevailed throughout December and January, but during the exceptionally warm February of 1957 winkle tracks were again apparent, although in nothing like the profusion noticed in the early autumn of 1956.

Observations of this kind suggest that winkles become inactive when the light intensity and temperature are low, and it is possible that these environmental factors of the winter act either singly or in combination. It is of interest in this connexion that Moore (1936) found that the growth rates of winkle populations in the Plymouth neighbourhood slowed down from October to February.

A few laboratory results will be quoted to show that both low light intensity and decreased temperatures lower the level of activity.

VARIATION IN RATE OF CRAWLING UNDER DIFFERENT LIGHT INTENSITIES

Individual winkles collected from horizontal surfaces and dark-adapted for 6 h were tested for rate of crawling under varying conditions of light intensity. These were obtained with an overhead light and a rheostat. The results are set out below. It will be seen that winkles behave orthokinetically in response to strong variations in light intensity, and it is likely that this has some importance in stimulating the animals to crawl and feed (Table 1).

TABLE 1. DISTANCE (CM) CRAWLED IN 5 MIN AT 19° C

Winkle number	At light intensity of 10 f.c.	3000 f.c.
1	22·0	27·0
2	22·5	30·0
3	17·2	24·3
4	16·0	17·0

VARIATIONS IN RATE OF CRAWL AT DIFFERENT TEMPERATURES

Individual winkles collected from horizontal surfaces and dark-adapted for 6 h were tested for rate of crawling at different temperatures (Table 2).

In each instance increase in temperature induced an increased rate of crawl. Further experiments were, therefore, carried out to determine the temperature at which winkles become immobilized.

Twelve winkles from vertical surfaces were placed in a dish of sea water at 0° C and illuminated from above at an intensity at the water surface of 10 f.c. All remained completely inactive for 20 min. Then the water was allowed to warm up gradually (see Table 3).

Twelve winkles from vertical surfaces were placed in a dish of sea water at 11° C illuminated from above at an intensity of 10 f.c. All began to crawl actively straightaway at a rate of about 2·5 cm/min. The water was then cooled gradually (see Table 4).

TABLE 2. DISTANCE (CM) CRAWLED IN 5 MIN

Winkle number	Temperature	
	12° C	25° C
1	10·5	17·8
2	10·4	15·7
	8° C	19° C
3	12·0	19·5
4	20·5	30·0
5	7·3	22·8
6	9·5	25·5

TABLE 3. EFFECT OF TEMPERATURE ON ACTIVITY

At	3° C	1 became active, i.e. put out its tentacles but did not crawl
	4° C	2 became active, i.e. put out their tentacles but did not crawl
	5° C	7 became active, i.e. put out their tentacles but did not crawl
	6° C	8 became active, and 3 of these began to crawl slowly
	8° C	12 became active, and 8 of these were crawling slowly
	10° C	12 were crawling at a rate of 2·5 cm/min

TABLE 4. EFFECT OF TEMPERATURE ON RATES OF CRAWLING

At	10° C	10 were crawling at a rate of about 1·5–2·0 cm/min
	8° C	8 were crawling at a rate of about 1·0–1·5 cm/min
	7° C	7 were crawling at a rate of about 0·5–1·0 cm/min

(the rest were inactive)

These results show that at temperatures of between 6° and 8° C winkles become inactive even when illuminated at light intensities sufficient to promote crawling at higher temperatures. They serve to explain why they remain largely inactive in the winter months from November to March when air and sea temperatures at Whitstable are usually below 8° C, although bright sunlight may occur at the time of low water. Indeed, observation on the shore makes it clear that low temperatures, rather than low light intensity, inhibit feeding migrations.

An interesting corollary of the relation between activity and temperature is that winkles which have settled on horizontal surfaces and which make their feeding excursions in the shallow layer of water covering them as the tide recedes may, in the warmer months of the year, experience temperatures well above those of the sea itself and so their feeding excursions are correspondingly longer, as can be noticed by a comparison of the tracks in the sand and on groynes.

DISCUSSION

In short, these observations made on the shore give the impression that winkles remain settled for a considerable part (probably the greater part) of each tidal period but that on occasions they undertake feeding excursions.

These excursions, as shown by tracks in the sand or on groynes, are reminiscent of the 'outward and homeward' journeys performed by many invertebrate animals, notably arthropods, but which are also known to be made very precisely by limpets (e.g. various species of *Patella*), less precisely by other shore gastropods, such as *Onchidium* and by chitons, as well as by some land-dwelling gastropods. Few of the homing reactions of intertidal gastropods have received detailed analysis and Thorpe (1956), commenting on the homing of limpets, remarks that: 'Apparent place memory has been observed in a number of other marine and intertidal gastropods, but the basis of it has not been properly investigated.'

The 'homing' reactions of winkles are, perhaps, comparable with those described and analysed by Evans (1951) for the small chiton, *Lepidochitona cinerea*, and evidently they tend to confine any particular winkle for a period of weeks or even months to an area of a few square metres, from which, however, it may become displaced by wave-action. Evans showed that at low tide *L. cinerea* is invariably found on the undersides of damp stones; that the animals move away from a strong directional light source but that they also have a strong positive orthokinetic response (so tending to accumulate in dark situations); that they are positively geotactic when out of water but are indifferent to the stimulus of gravity when immersed, and that a combination of these reactions to common stimuli serves to explain their ability to shelter under stones at low tide, whilst at high water allowing them to carry out feeding migrations. The rather limited excursions of these small chitons receive, then, explanation without recourse to 'place memory'. But, bearing in mind the more varied situations in which winkles are to be found at the time of low water, it is apparent that their reactions are likely to be less stereotyped than those of *Lepidochitona* although their feeding migrations may still be orientated by responses to light and to gravity. Thus, the vertical U-shaped tracks made by those which settle either on the east or west faces of groynes in regions of light or shade, exposed or in cracks, cannot, it would seem, be the result of movements orientated solely with respect to the direction or intensity of light. Rather they suggest excursions determined by gravity responses. On the other hand, it is inconceivable that the tracks made on the virtually horizontal surface of sandy patches owe anything to gravity responses. Indeed, the only directional stimulus would seem to be that of light from the sun to which at least one marine mollusc, viz. the opisthobranch *Elysia viridis* is known to orientate by performing a light-compass reaction (Fraenkel, 1927). A more detailed review of the literature on the behaviour of winkles is given in the article that follows in this Journal (p. 241).

SUMMARY

On the flat shore at Whitstable the population of common winkles is distributed appreciably at random over a variety of substrata ranging from wooden groynes, through shingle to muddy sand, between tidal levels corresponding to H.W.N.–E.L.W.S. but with a maximum density near M.T.L., although aggregations tend to occur in wetter situations and where planking joins vertical groyne posts.

Observations on winkles marked with a dab of paint of distinctive colour show that they tend to remain in approximately the same position for many weeks.

For most of each tidal period winkles remain settled in situations such as stones or groynes, which provide good holding ground, but as the tide recedes or the incoming tide reaches them, they become activated to crawl, feeding meanwhile. These feeding migrations are recorded in the sand by roughly U-shaped tracks and can be seen under favourable circumstances as similar tracks in the vertical plane for winkles on groynes. By such excursions the animals are enabled to move, feed and return to approximately the place from which they started and this serves to explain how they maintain their station on the shore.

When settled on vertical surfaces, winkles always orientate with the head uppermost, when the foot may be withdrawn and the animal become attached by a film of dried mucus.

When crawling the eyes are exposed and the tentacles bend to test the substratum, but only that part of the tentacle distal to the eye bends so that the optical axis of the eye maintains a constant angle to the body axis.

Winkles are inactive during the winter and laboratory tests show that crawling ceases at about 8° C. The animals also behave orthokinetically in response to strong variations in light intensity, crawling faster at higher light intensities.

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AN EXPERIMENTAL ANALYSIS OF THE BEHAVIOUR OF *LITTORINA LITTOREA* (L.) UNDER NATURAL CONDITIONS AND IN THE LABORATORY

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(With Text-figs. 1–10)

The previous paper (Newell, 1958) was mainly an account of straightforward observations of the winkle population on the shore at Whitstable. These suggested additional work on the activities of the animals, particularly their responses to light and to gravity, which are important in the maintenance of their position on the beach.

RESPONSES TO SUNLIGHT ON THE BEACH

The fact that individual tracks in the sand seem to follow an apparently orientated course suggests that winkles are reacting to visual clues, since the terrain is flat and featureless. To test this supposition a winkle crawling from east to west with the sun on its left-hand side was shaded from the sun by a piece of cardboard. Then the image of the sun was shone on its right-hand side by means of a plane mirror. Immediately the winkle changed direction and crawled back on a track parallel to its original course. This experiment was repeated with several other specimens, some of which were moving from approximately north to south and in various other directions, but with the invariable result that their direction of crawling was reversed when the apparent direction of the light was altered. A slightly different experiment was one in which winkles were lifted from the sand and placed on a moist plate. Most began crawling in more or less straight lines and when the plate was turned through 180 degrees the direction of crawling was reversed, each winkle countermarching approximately along its original path.

These results seem to show that winkles crawling on wet sand are orientating to the direction of the sun in a manner which seems likely to be a 'light-compass reaction' (Fraenkel & Gunn, 1940), since, whilst individuals pursue an orientated course for some time, yet the direction of crawling is capable of reversal and, moreover, there is a good deal of variation in the direction of movement as between individual winkles. The maze of trails in the sand, despite the statement of Schwarz (1932), gives no immediate impression of constancy of direction for the population as a whole and the discovery that each trail is, in fact, a record of orientated crawling was made possible only because attention was focused on small instead of large samples of animals. Nevertheless, it does not follow that there is no pattern of orientation for all the winkle trails even if it is not at first sight obvious.

A survey made at 13.30 to 14.30 h on 14 October at the time of low water, roughly 4 h after the tide had uncovered the area, showed the usual maze of tracks in the sand, but only a few winkles were still crawling. The bearing of 125 of these tracks was measured to the nearest 10 degrees (magnetic) by means of a prismatic compass and the results are given in the form of a polar diagram (Fig. 1 A). From this it is at once obvious that the majority of tracks are approximately towards and away from the sun but that there is a good deal of variation and 15%, approximately, are at right angles to the sun's rays. It must be remembered, however, that the tracks are not all made at the same time even at one tidal level, and, as the tide recedes, the apparent motion of the sun will cause tracks on lower levels to differ in direction from those higher up the shore with the result that the records made on any survey will be expected to show some 'scatter'. Nevertheless, polar diagrams (Fig. 1 B–D) similar to that in Fig. 1 A, but made from tracks recorded at different times of the day and for different tides in the lunar cycle from neaps to springs, fully confirm the idea that winkles crawl mainly along the direction of the sun's rays, whilst observations as the tide recedes show that at first the direction of crawling is mainly towards the sun and later, mainly away from it. Fig. 1 D, although very similar to the three previous diagrams, was constructed from results obtained on a dull summer day and suggests, therefore, that the animals were orientating to the brightest patch in the sky. It remained possible, however, that the pattern of polarized light in the sky might be providing the visual cue to which the responses were made. To test this animals were completely shaded by a large sheet of 'Polaroid' which was rotated to varying degrees and the effect on the direction of crawling noted. Of fifty winkles tested in this way none showed any clear-cut alteration of the direction of movement, most of them continuing to crawl in the same direction as when they were fully exposed.

Similarly, winkles tested in the laboratory with a sheet of 'Polaroid' interposed between them and a 200 W. lamp, also seemed not to orientate to the plane of polarization. These observations are, admittedly, insufficient to state definitely that winkles do not orientate to the plane of polarization but, even if further experiments show that this can affect their direction of crawling under laboratory conditions, it is unlikely that responses of this kind play any part in the lives of animals in natural conditions, a point of view clearly expressed by Stephens, Fingerman & Brown (1953) in a discussion of the orientation mechanism of *Drosophila*. These authors also point out that, as expressed by Fresnel's Laws, at all angles of incidence except 0 and 90 degrees, 'light vibrating in the plane defined by the incident and reflected rays will be more efficiently refracted than light vibrating in a plane normal to this'. This applies even to light entering a non-birefringent medium, and so an apparent response to the plane of polarization of the light may be an indirect one, the animal in reality responding to the amount of light entering the eye.

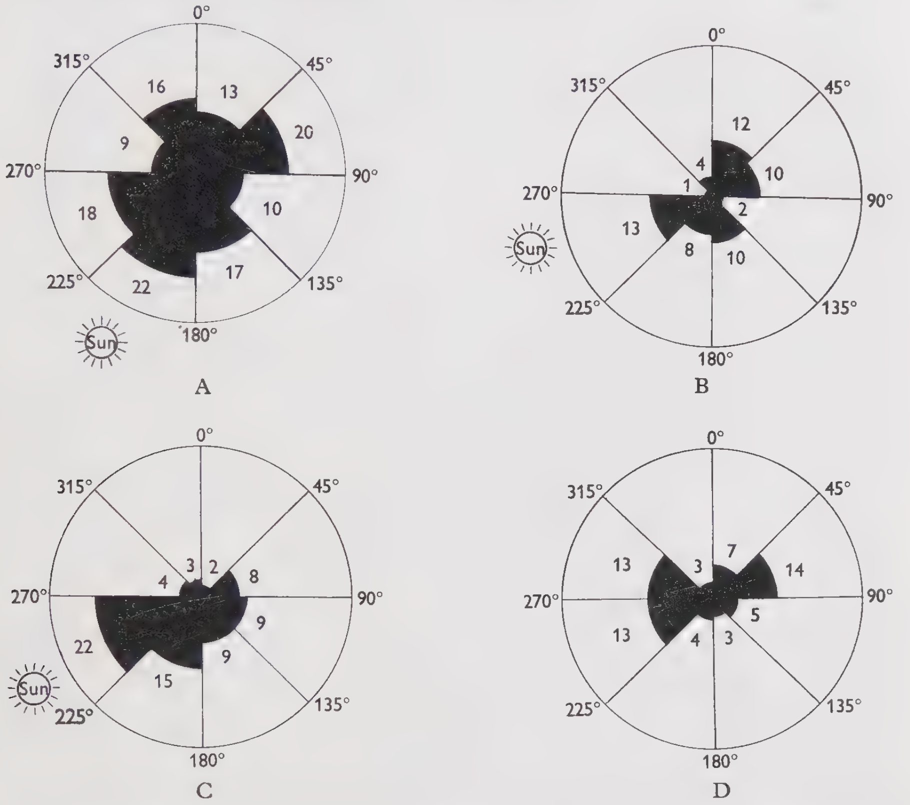

Fig. 1. Polar diagrams to show the directions of tracks and numbers of winkles moving along various tracks in the sand. Bearing measured to the nearest 10° magnetic. (A) 14 October 1956, bright sunlight, low water, neap tides; (B) 25 March 1957, 5 h after low water, sun shining only occasionally between clouds; (C) 27 March 1957, 1 h after tide had receded from M.L.T., sun's disc showing through faint haze; (D) 31 July 1957, low water, overcast sky, position of sun, 290°.

BEHAVIOUR OF WINKLES IN AN AQUARIUM

If winkles are collected at random from a variety of situations on the shore which include vertical, sloping and horizontal surfaces, their behaviour in an aquarium is also very variable. Soon after being tipped in, practically all of them begin to crawl about, but this activity is not continued indefinitely. After some hours of what appears to be random crawling, they settle down, some on the sides at varying heights relative to the water-level and some on the bottom. In these situations the vast majority remain motionless for hours or days but very occasionally one will become active and crawl about. Here again there is no uniformity in the direction of crawling.

After being kept in captivity for periods of 2 weeks or longer, all the winkles, even those which have settled below the waterline, become completely inactive. Many of those settled on the sides fall off and lie upside down on the aquarium floor with the head and foot in varying states of extension. It might be thought that lack of food or some adverse feature of the aquarium conditions causes winkles to become moribund after a time, but observations show that this is not so. For instance, any of these apparently moribund animals will contract immediately into its shell if picked up and will begin to crawl actively if placed on a suitable surface.

From this it appears that the winkles are quite healthy but do not behave normally after a time in captivity. Yet their behaviour is not totally different from that observed in natural populations. Rather, it appears distorted and in an aquarium with sand and shingle on the floor, with well-oxygenated water and a supply of food, the chief variant from the natural habitat would appear to be that there is no up-and-down oscillation of the water-level corresponding to the tides.

Winkles which have settled on the vertical sides or bottom of any vessel can be stimulated to begin crawling by filling it with water. Moreover, immersion is not the only adequate stimulus, since shaking or sudden increase in light intensity will usually suffice. The immediate response is always a sudden withdrawal into the shell, but crawling is soon resumed.

One is now reminded of what has been observed on the shore and is in a position to formulate a provisional hypothesis along the following lines. In the absence of stimulation, winkles tend to become inactive and cling to the nearest available surface; when stimulated, as by immersion by the rising tide and jolting by wavelets, they begin to crawl and feed; but when immersed and under uniform conditions they cease moving after a time and settle down. Clearly those on horizontal surfaces, and especially on wetter parts of the shore, will experience different conditions from those settled on groynes. The available evidence suggests that the stimulus to crawl on flat surfaces is an increase in light intensity and jogging by the breaking wavelets as the tide falls. Winkles on vertical surfaces are similarly activated but they are also induced to settle by desiccation, which in its extremer conditions causes withdrawal of the foot and adhesion solely by means of hardened mucus (Wilson, 1929).

REACTIONS TO GRAVITY

So far, attention has been paid only to winkles crawling on the virtually horizontal surface of wet sand, but an appreciable proportion of the population lives, often densely aggregated, on the wooden groynes running at right angles to the shoreline from H.W.S. to a varying distance below H.W.N. Tracks made by the periodic feeding excursions are not easy to follow in these situations, but, as has been mentioned, on some groynes tracks can be seen to be made up of a downward, horizontal and upward limb. They thus resemble in general form those made on horizontal surfaces, but since winkles occur on either the west or east face of groynes, in light or in shade, it would seem unlikely that the movements are orientated solely with respect to light but rather that gravity provides the necessary directional stimulus.

A chance observation is of interest in this connexion and serves also to reinforce the idea that the clue to an understanding of the behaviour is to pay regard to individual winkles, taking note of the exact situation from which they were collected. A batch of winkles was collected from the top surfaces of flat stones and a similar batch from the vertical face of a groyne. Each batch was placed in a separate tin and after 2 h those from flat surfaces, without exception, were still at the bottom of the tin, whereas those from the groynes were settled on the sides of their tin, clear of the bottom. This shows that those from horizontal surfaces are indifferent to gravity, whereas those from vertical surfaces behave, at least for a time whilst in the dark, in a negatively geotactic fashion. This observation has been confirmed on six subsequent occasions. On one of these, namely on 26 September 1956, the winkles were examined at intervals of 2, 6, 12, 16 and 72 h. Those from vertical surfaces remained settled on the sides of the tin, whereas those from horizontal surfaces were all massed at the bottom. It remains to be seen if winkles from vertical surfaces are indifferent to the stimulus of directional light, but this can be tested only under laboratory conditions (p. 255).

As has been mentioned winkles react to the stimulus of gravity in a variable manner according to the slope of the position on which they have settled. It is this which accounts for variations in behaviour in an aquarium when animals are collected at random from the shore. To eliminate this variable, a batch of twelve winkles was taken from the strictly horizontal surfaces of flat stones left high and dry by the tide. Each animal was marked with a dab of yellow paint. A second batch was collected from the tops of flat stones in a pool of water and marked with blue paint whilst a third batch from vertical surfaces was marked with a spot of red paint. Each of these batches of twelve winkles was placed in an aquarium with sand and shingle in the bottom, the larger stones projecting above water-level. After crawling and feeding for some hours, all the animals settled down.

A summary of their subsequent movements is set out below, from which it will be seen that those collected from flat surfaces tended to settle in somewhat similar situations, irrespective of whether they were dry or not, whereas those from vertical surfaces tended to settle on the sides of the aquarium, i.e. on vertical surfaces. This confirms previous findings, viz. that the past history of a particular winkle influences its geotactic response.

Twelve winkles from flat, dry stones, marked with yellow paint. None climbed up the tank in the first hour. After 2½ h four were on the sides at the surface film and the rest on stones lying on the bottom of the tank. After 3 days seven were on stones and five on the sides of the tank at the surface film.

Twelve winkles from flat, submerged stones, marked with blue paint. None climbed up the sides of the tank in the first hour. After 2½ h two were on the sides of the tank but below the waterline. After 3 days six were on stones, four were on the sides of the tank and two on the bottom.

Twelve winkles from vertical surfaces marked with red paint. Twelve climbed above the waterline in 6, 8, 9, 11, 14, 15, 17, 22, 24, 25, 25 and 31 min respectively. After 2½ h eight animals had moved down and settled at the waterline, head uppermost. After 3 days, nine were settled on the sides and three were crawling on the bottom of the aquarium.

Results confirming those set out above were obtained by collecting winkles from the vertical faces of groynes and from the tops of flat stones and testing their geotactic responses in separate vessels. For example, twelve winkles from groynes were placed in a large bottle full of sea water. Six immediately crawled up and reached the top in 2 min. They stayed there until accidentally dislodged. Three crawled up and settled on the sides but below the waterline within 2 min, whilst after 2 min the remaining three were still crawling on the bottom. This experiment was made in diffuse light but essentially the same result was obtained in bright sunlight. Varying the direction of the light rays entering the bottle appeared to have no effect on the behaviour of the animals. The bottle was then put in complete darkness and after 2 h seven were at the top, two were on the sides but just below the waterline and three were on the bottom.

This seems to show that winkles from the sides of groynes are predominantly negatively geotactic; but subsequent observations show that this view, which accords with most previous workers, requires modification, for, as will be seen, under suitable conditions winkles reverse the sign of their response, and it is this reversal which accounts for the lack of a 100% uniformity in the previous experiment.

Twelve winkles collected from horizontal surfaces were placed in a small glass tank in diffuse light. All began to crawl but repeatedly turned as if unable to orientate. After 4 h, although apparently random movements brought them into contact with the sides of the tank, none had climbed the walls. Thus, winkles from flat surfaces are not negatively geotactic, at least for some time. Yet, after 24 h in the tank all the winkles of this same batch had settled on the waterline.

It can, therefore, be assumed that all winkles have a tendency to climb and settle on vertical or steeply sloping surfaces, but that if this opportunity is denied them, the response becomes abolished for some time, which other experiments show may be as long as 10 days. This seems to be a process of habituation as defined by Thorpe (1956).

GRAVITY REACTIONS OF WINKLES AFTER DRYING

Bearing in mind that all winkles settled intertidally on vertical surfaces experience a semi-diurnal drying after the tide has receded, it seemed important to test if drying has any effect on the responses to gravity. Therefore, instead of beginning observations, as in previous experiments, as soon as possible after collecting the animals, time was allowed for them to dry.

Twelve winkles collected from groynes at the time of low water were left in a tin for 5 days. Then, three at a time, they were allowed to settle on a horizontal moist glass plate. This was then placed vertically in a large beaker of sea water. Immediately all the winkles began to crawl *downwards*. That is, contrary to previous occasions, they behaved *positively* geotactically. At levels of from 3 to 5 cm below the surface they moved about more or less horizontally and then crawled up to settle at or above the waterline. This was the invariable result and shows that after a period of desiccation winkles behave positively geotactically, are then neutral to the stimulus of gravity for a time and finally reverse the sign of their response. Light can be ruled out as an essential orientating stimulus since the experiments were carried out in diffuse light whilst exactly comparable results were obtained with light from directly above the beaker or shining through the sides.

Additional results are set out in tabular form (Table 1).

TABLE 1

Winkle number	Time in minutes under water
1	7
2	9
3	10
4	15 (but then returned for 4 min)
5	15
6	12
7	72 (fed on *Ulva* until it was all gone)
8	6
9	12
10	9
11	9
12	7
13	11
14	6
15	3
16	3
17	settled at waterline
18	6

The results of the previous experiment have an obvious bearing on the interpretation of the behaviour of winkles under natural conditions on the beach groynes, since they clearly point to the conclusion that the animals settle down in the head-up position but become positively geotactic after a period of desiccation. This enables them to carry out feeding migrations when reached by the incoming tide. A reversal of the gravity response, so that its sign becomes negative, causes them to return approximately to the position they had left, when they settle down and later on experience yet another period of drying...and so on. One is reminded of Mitsukuri's results on *Littorina exigua* (p. 259) which showed that this winkle also moves downshore after a period of desiccation, although Mitsukuri believed that this was because they became positively phototactic.

It was obviously of interest to repeat the previous experiment on winkles which had not been desiccated for a period of as long as 5 days. A summary of the results of a further series of tests is set out below.

Eight winkles were collected from the vertical face of a groyne at 09.00 h at approximately low water and allowed to settle on a moist glass plate at 10.30 h. The plate was then placed vertically in a beaker of sea water in diffuse light. Each specimen moved down, then moved horizontally and finally returned to the waterline, the times for these migrations being as follows (Table 2).

TABLE 2

Winkle number	Time in minutes of excursions
1	10
2	6
3	15
4	12
5	10
6	15
7	55 (fed on a piece of *Ulva* until it had all gone)
8	9

This experiment confirms previous results and also shows that a period of desiccation of about 1½ h is sufficient to initiate what must now be regarded as the normal tidal feeding excursion. Here, as in the previous series, one winkle stayed below water for a much longer time than any of the others, owing, it would seem, to the chance finding of food. Thus the behaviour of winkles collected from vertical surfaces becomes predictable in the laboratory under conditions which simulate those on the beach. They become active after immersion and crawl, first downwards, then horizontally and then upwards, attempting to feed meanwhile.

That feeding does, indeed, modify the duration of the feeding excursion is shown by the following experiment.

A batch of ten winkles was divided into two equal groups and each was placed in a tank with only 1 cm water in the bottom. One batch was allowed to feed for 2 h on *Ulva* whilst the other batch was left unfed. Each batch was then immersed by running in sea water. The results are set out in Table 3. This clearly shows that fed winkles are less active than unfed ones and that the presence of food prolongs the period of immersion.

TABLE 3

Fed	Unfed
None moved at once. Four stayed immersed for $1\frac{1}{2}$ h. After 3 h, four had climbed above the waterline but one remained below it still feeding.	All became active at once and all five crawled down, then horizontally and then up above the waterline in 45 min.

Another experiment which gave a more clear-cut result was carried out on winkles which had been collected 10 days previously from a groyne. From these, two batches of five each were taken at random. One batch was allowed to feed on lettuce for 2 h. The other batch had been starved during the 10 days' captivity. Each batch was then allowed to settle on the sides of a jar with a little water in the bottom. All settled above the waterline. The experiment began when water was run into the jars so as to submerge all the winkles. The results are summarized below.

Fed winkles. All five animals were near the top of the jar. When submerged none became active for some minutes and none crawled about under the water. After 35 min all had crawled up out of the water.

Starved winkles. One winkle was near the top of the jar and four on the sides lower down. All became active immediately after immersion, crawled down, then horizontally and finally up above the waterline in times which varied from 7 to 38 min.

This not only confirms the view that fed winkles are less active than starved ones, but shows also that feeding abolishes the positively geotactic behaviour which is always evoked by the immersion of starved winkles. The longer times of the feeding migrations of these winkles which had been starved for 10 days, when compared with those carried out on animals kept in captivity for shorter periods suggest that the time spent in an attempt to feed is related to the degree of starvation.

The experiments described so far have indicated that the behaviour of winkles collected from the shore at Whitstable varies a good deal according to the situation on which the animals have come to rest, but observations on gravity responses have been chiefly on winkles collected from vertical surfaces. Comparable experiments on those from horizontal surfaces give different results, as is shown, for example, by those summarized below.

Twelve winkles were collected from the tops of flat stones at 09.00 h at low tide and were tested for gravity responses at 11.30 h. Each winkle was placed on a moist glass plate. After settling, the plate was placed vertically in a beaker of sea water illuminated by diffuse light from a north window. Each winkle was, in the first instance, placed so that it was orientated transversely across the plate. Most stayed in this position but four re-orientated to assume the head-up position directly the plate was tipped up so that it looked like a mechanically caused slip, and three out of these four animals later orientated across the slope. Three slipped off the plate but none crawled up or down.

These observations show that winkles from horizontal surfaces are, indeed, as has been suggested by previous experiments, indifferent to the stimulus of gravity.

So far the gravity reactions of winkles have been tested only in response to strictly defined surfaces, but on rocky shores winkles will experience slopes intermediate between the horizontal and the vertical. It is, therefore, of interest to find the minimum slopes which will evoke geotactic responses. This was done by placing winkles on horizontal surfaces and then tilting up the plate, 10 degrees at a time. As has already been seen, winkles from horizontal surfaces pay no regard to the stimulus of gravity and so only animals from groynes were tested in this manner. Several experiments of this nature were carried out. Practical difficulties arise. For example, not all the winkles used adhere to the plate and so some fall off when the plate is tipped at angles of 40 degrees to the horizontal or greater; some settle down and adhere but fail to crawl; others crawl horizontally and then off the plate and up the sides of the containing vessel.

Twelve winkles collected from groynes at time of low water were placed on a wooden plate with their heads downwards in diffuse light. The plate was then tipped up 10 degrees at a time and left on each occasion for 2 min. The minimum angle at which any winkles orientated to the head-up position was 10 degrees.

Twelve winkles collected from groynes at low water were placed with the longitudinal axis across the plate which was then tipped up, 10 degrees at a time. The plate was kept moist but was not immersed in water. From Table 4 it will be seen that at the lower angles winkles tended to retain the orientation at which they were placed on the plate, but that at 90 degrees all twelve assumed the head-up position, either statically or by crawling up.

TABLE 4

Angle (degrees)	Orientation		
	Up	Down	Horizontal
10	3	2	7
20	3	4	5
30	2	3	7
40	3	3	6
50	2	2	8
60	2	0	10
65	7	0	5
70	7	1	4
80	11	0	1
90	12	0	0

All that can be inferred from the results is that the minimum slope to which winkles can orientate is probably about 10–20 degrees, that at slopes of 60–65 degrees most of them assume the head-up position and that at 90 degrees all winkles orientate head-uppermost. This is rather different from saying that at steep angles winkles tend to behave in a negatively geotactic fashion, since many animals remained in the same position although re-orientating with respect to gravity. Indeed, in other experiments some of the more active specimens crawled right to the top of the vessel and then over the top and *downwards* on the outside surface.

Attempts by previous workers, as for example Kanda (1916), to assess the strength and nature of the response of winkles to gravitational stimuli gave conflicting results, and from what has been said it will be realized that this can be attributed to two main causes. In part it could have been due to a failure to appreciate that winkles collected from steeply sloping surfaces normally alter the sign of their response to gravity after crawling for some time, so that intermittent observations might catch a winkle during its downward, horizontal or upward phase of movement. It is also true that previous workers paid insufficient attention to the exact situation from which winkles were collected, so that those from sloping surfaces were mixed with those from horizontal ones, when, of course, a variety of responses was detected. Gowanloch & Hayes (1926) showed that, statistically, winkles from higher levels on the shore tended to crawl upwards more strongly than those from lower levels, the implication being that in some way this difference in behaviour was related to the position on the shore, but they paid no regard to the winkles as individuals. It seems likely, however, that the differences they noted are due, not to a difference in shore levels or zones as such, nor to time of exposure to air, etc., but, instead, to a difference in beach slope at various positions relative to tide marks. I am indebted to Mr Derek Mills for the information that at New Brunswick (where Gowanloch & Hayes made their collections), the shore has a steep upper beach and a more or less horizontal rock platform near low water-level, so that the differences in behaviour with respect to the stimulus of gravity may be attributed to habituation to a horizontal or steeply sloping surface, such as has been noticed for the winkle population at Whitstable.

Summary of results of observations on the reactions of winkles to gravity

(1) Winkles have an inherent tendency to settle on vertical or steeply sloping surfaces, when they assume the head-up position.

(2) Provided that the surface is moist, when stimulated to crawl the animals move first downwards, then horizontally and then up again, to regain approximately their original position. Immersion in water, mechanical agitation or a sudden increase in light intensity are stimuli which are adequate to promote crawling.

(3) The excursions from the position of settlement can be regarded as feeding migrations.

(4) Winkles collected from horizontal surfaces are indifferent to gravitational

stimuli for periods which vary from some hours to 10 days. They have become habituated to flat surfaces but this habituation gradually diminishes, so that when presented with an opportunity to crawl upwards they revert to their inherent pattern of response.

(5) Fully fed winkles do not respond by crawling downwards when stimulated to move, but starved winkles make longer feeding excursions below water if they happen to find food.

(6) The minimum slope to which winkles orientate is about 10–20 degrees.

REACTIONS TO LIGHT

Winkles collected from horizontal surfaces

The following observations are a selection from very many which were made and serve to show that winkles from horizontal surfaces react to the stimulus of light in a way which is consistent with the form of the tracks seen on the sand at low tide.

Twelve winkles were taken from the tops of flat stones shortly before the incoming tide reached them but no note was taken of their exact orientation on the stones. The animals were placed in a shallow glass dish of sea water in bright sunlight, the sun being nearly due south.

The animals began to crawl almost at once. Seven crawled away from the sun and five towards it. Not all the paths were directly towards or away from the sun but any obliquity of the paths brought the winkles to the sides of the dish, when, of course, the paths of necessity became straight. After 3 min seven were at the end away from the sun and five at the end nearest the sun. After about 5 min three animals which had formerly been photopositive became photonegative and crawled to the opposite end of the dish, whilst the two which were photopositive became photonegative. This reversal of response was confirmed by turning the dish through 180 degrees, when the five winkles under consideration also turned round and maintained the same orientation to the sun's rays. This chance observation also confirms what had been seen only rarely on the beach but which had been inferred from the form of the tracks in the sand, viz. that winkles on flat surfaces reverse the sign of their response to light. From this it is also obvious that the immediate past history of an animal will modify the results obtained when testing its light reactions, and winkles collected at random from horizontal surfaces would not be expected to display any uniformity of orientation to light. Indeed, the result set out below is typical of many which were obtained even when winkles were collected from exactly similar situations.

Twelve winkles were collected from horizontal surfaces, all with their heads facing the sun, and placed in a shallow dish of sea water. Three behaved photopositively, three behaved photonegatively, and six moved across the sun's rays.

The experiments, an account of which follows, were designed to test the reactions to light of winkles which had received uniform treatment for some time before the observations.

Eight winkles from horizontal surfaces were kept in a tin for 48 h and then placed in a shallow dish with the bottom and three sides covered by black paper. A beam of parallel light was shone through the uncovered end of the dish from a microscope lamp. Two winkles crawled towards the light; after 2 min two winkles crawled away from the light, four winkles crawled transversely; and after 6 min eight winkles crawled towards the light. This shows that unfed, dark-adapted winkles are positively phototactic, but that there is some variation in the initial direction of their crawling.

In order to record the paths followed by winkles crawling when illuminated by a parallel beam of light, animals which had been dark-adapted for 4 days were placed in a large developing dish whose walls were covered with black paper and which was floored with cm graph paper. Light beams from microscope lamps gave an illumination of 250 ft. candles at the floor of the dish and could be shone either at right angles or from opposite ends of the dish. The movements of the winkles were recorded at half-minute intervals on another piece of graph paper.

The selection of tracks which follow (Figs. 2–5) shows that all the winkles behaved positively phototactically but that when two lights were shone at right angles a crawling animal usually disregarded the second light and continued to crawl towards the first one. This suggests that they are behaving telotactically (Fraenkel & Gunn, 1940), a type of behaviour which has much in common with light-compass reactions.

In a repeat of the above experiment twelve dark-adapted winkles collected from horizontal surfaces were placed in a dish of sea water lined by black paper. A beam of parallel light was shone in at one end. All were strongly photopositive for 12 min but at times varying from 12 to 20 min they became photonegative. This, it will be realized, confirms observations made previously (*a*) on the shore, and (*b*) in a dish using the sun as a light source. But such clear-cut results were not always obtained. On some occasions winkles reached the sides of the dish before finally orientating towards or away from a light source and then the direction of crawling in part became determined by the sides of the dish, for the winkles move along the line of junction of sides and floor.

Winkles collected from vertical surfaces

It has already been shown that winkles from vertical surfaces make feeding excursions during which they orientate with respect to gravity, but this does not preclude the possibility of reactions to light entering into their responses. Their place of settlement, however, introduces a factor quite different from that affecting winkles on horizontal surfaces, for, since the groynes are opaque,

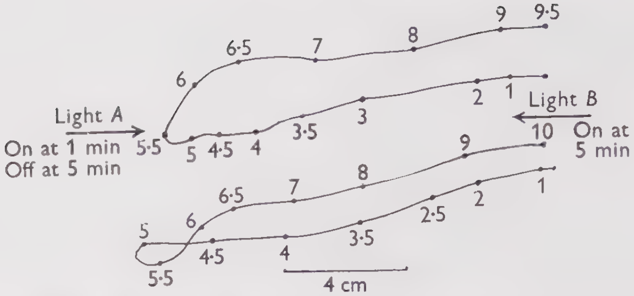
Light A
On at 1 min
Off at 5 min
Light B
On at 5 min
9
9·5
6·5
7
8
6
2
1
3
5·5
5
4·5
4
3·5
10
9
8
7
6·5
6
5
4·5
4
3·5
2·5
2
1
5·5
4 cm

Fig. 2. Tracks of two winkles collected from horizontal surfaces and dark-adapted for 4 days. Numbers along tracks indicate time intervals in minutes. Lights *A* and *B* are microscope lamps.

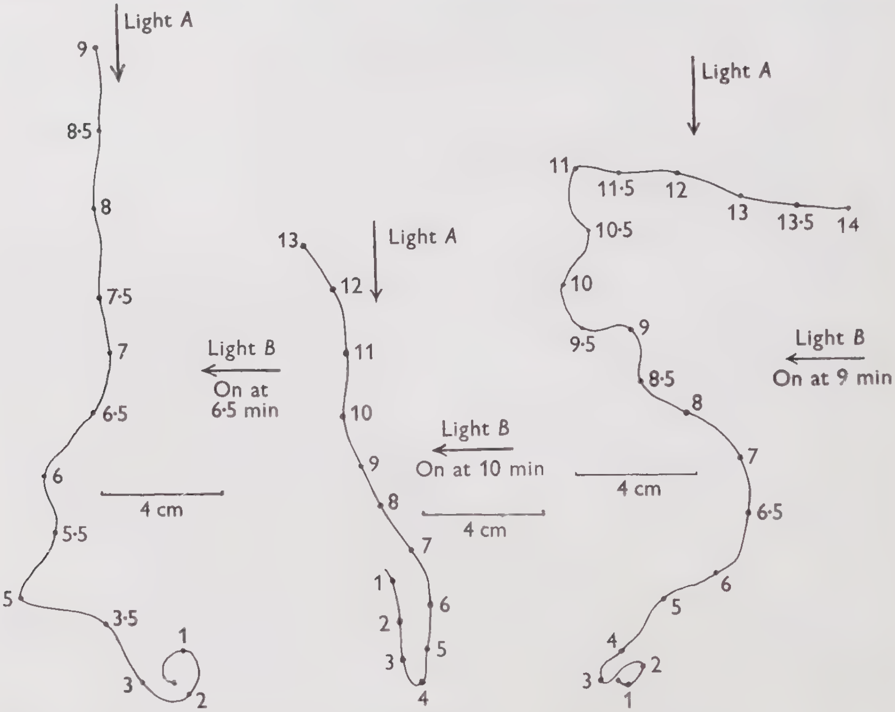
Light A
Light B
On at 6·5 min
4 cm
9
8·5
8
7·5
7
6·5
6
5·5
5
3·5
3
2
1
Light A
Light B
On at 10 min
4 cm
13
12
11
10
9
8
7
6
5
4
3
2
1
Light A
Light B
On at 9 min
4 cm
11
11·5
12
13
13·5
14
10·5
10
9·5
9
8·5
8
7
6·5
6
5
4
3
2
1

Fig. 3 Fig. 4 Fig. 5

Figs. 3-5. Tracks of winkles collected from a horizontal surface and dark-adapted for 4 days. Numbers along tracks indicate time intervals in minutes. Lights *A* and *B* are microscope lamps.

the incident light must always be mainly from above. It will, however, vary in intensity as the tide rises and falls. When covered to the depth of a metre or more by the turbid water at Whitstable, winkles will be almost in darkness, since even a depth of 14 cm of water cuts out 95% of the light reaching the surface (Aleem)[1] and they will become dark-adapted during a period of some hours. As the tide falls the light intensity increases and their behaviour might be expected to vary in a way comparable to that noticed for winkles from horizontal surfaces, viz. they might reverse the sign of their response to light after some time of exposure to higher intensities of illumination. In order to rule out gravitational responses the reactions to light of winkles collected from vertical surfaces were tested by observing their crawling reactions on the floor of a dish.

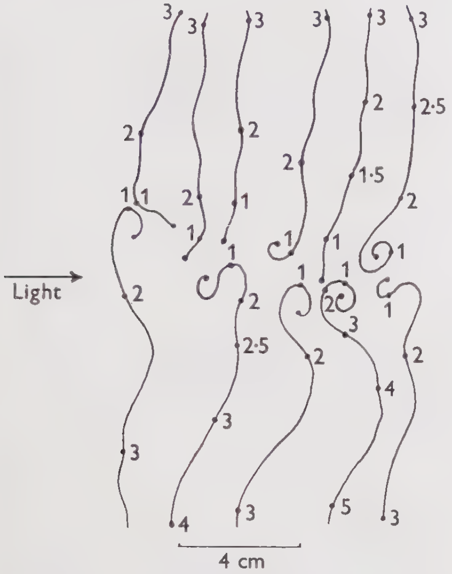

Fig. 6

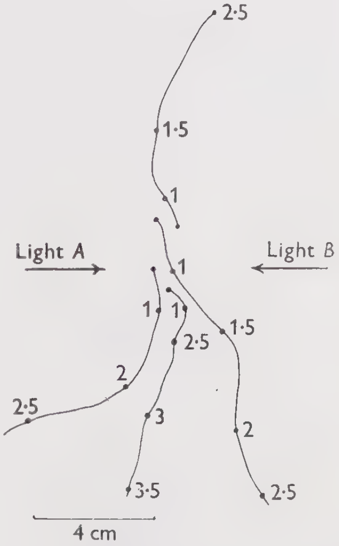

Fig. 7

Fig. 6. Tracks of winkles collected from vertical surfaces and kept in diffuse light for 4 days. Numbers along tracks indicate time intervals in minutes. The light is from a microscope lamp.

Fig. 7. Tracks of winkles collected from vertical surfaces and dark-adapted for 4 days and then exposed to diffuse light for 2 h. Numbers along tracks indicate time intervals in minutes. Lights *A* and *B* are microscope lamps.

Numerous observations on the reactions of winkles from vertical surfaces kept in diffuse light for periods of up to several days showed that the animals, when placed in a dish of sea water and illuminated by a beam of parallel light from a lamp, crawled mainly across the light beam. A selection of tracks made under these conditions in given in Figs. 6 and 7.

Fully dark-adapted animals behave somewhat differently. At first most animals crawl away from the light source, but after some time of exposure to illumination they orientate across the light beam, and later still some become photopositive. Some of these responses are shown in Figs. 8–10.

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[1] University of London, Ph.D. Thesis, 1948.

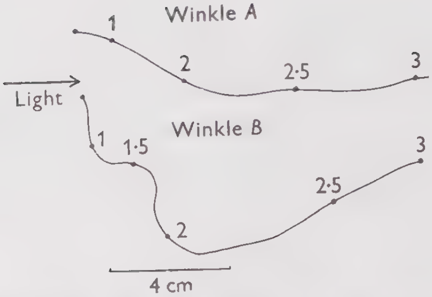
Winkle A
1
2
2·5
3
Light
Winkle B
1
1·5
2
2·5
3
4 cm

Fig. 8. Tracks of two winkles collected from vertical surfaces and dark-adapted for 4 days. Numbers along tracks indicate time intervals in minutes. The light is from a microscope lamp.

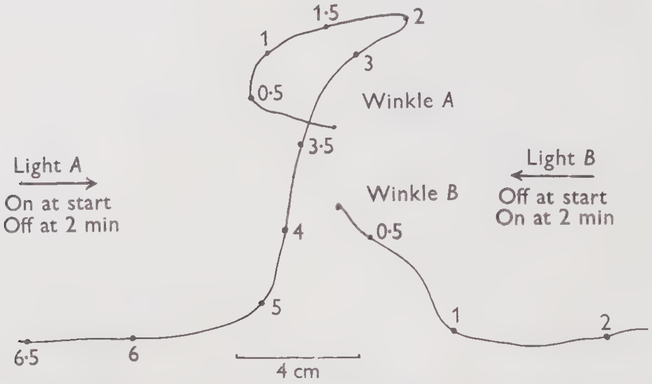
1·5
2
1
3
0·5
Winkle A
3·5
Light A
On at start
Off at 2 min
Winkle B
Light B
Off at start
On at 2 min
0·5
4
5
1
2
6·5
6
4 cm

Fig. 9. Tracks of two winkles collected from vertical surfaces and dark-adapted for 4 days. Same two winkles as in Fig. 8. Numbers along track indicate time intervals in minutes.

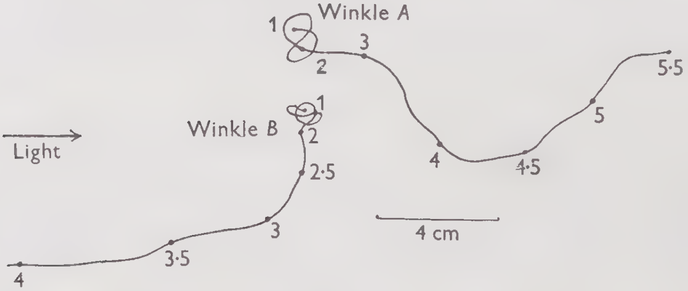
Winkle A
1
3
2
5·5
1
Winkle B
2
Light
5
2·5
4
4·5
4 cm
3
3·5
4

Fig. 10. Same two winkles as in Figs. 8 and 9. Winkle *A* remained photo-negative but winkle *B* became photopositive after 2 min and remained so when repeatedly retested.

In addition, it should be mentioned that of fifteen winkles collected from vertical surfaces and dark-adapted for 8 h, thirteen were at first photonegative, one was photopositive and one crawled across the light beam. Six which were at first photonegative reversed their direction of crawling after 5 min.

Thus, the feeding migrations of winkles on vertical surfaces are probably, in part, determined by their responses to light. When dark-adapted the animals behave photonegatively, but after a time they orientate across the light beam and they then crawl upwards, having reversed the sign of their response to light. In contrast to winkles from horizontal surfaces, those from vertical surfaces are at first photonegative and their feeding migrations are determined in part by gravity and to a lesser extent by responses to light. Further light is thrown on this point by the observations which follow and which were made to see how winkles behaved when (*a*) the light stimulus is removed, and (*b*) when it is acting against gravity.

(i) Dark-adapted winkles from horizontal surfaces were allowed to settle on horizontal glass plates in darkness, when they orientated at random. Viewed in ruby light they crawled, when stimulated by immersion, in a very sluggish fashion and within 5 min had settled again.

(ii) When twelve dark-adapted winkles from vertical surfaces were allowed to settle in darkness on a vertical glass plate in an empty beaker, all crawled upwards and settled. In ruby light all at first crawled down and then up above the waterline when the beaker was nearly filled with sea water. This shows that in darkness all the winkles when stimulated crawled downwards but that all behaved in a negatively geotactic manner before settling down, although the downward limb of the track was short and the crawling was sluggish.

(iii) The experiment was repeated but with the difference that, when the winkles were submerged, illumination from directly below was provided by a 75 W. lamp. Then eight winkles crawled horizontally, five crawled upwards, two crawled downwards and then up. All settled just above the waterline after 12 min.

This shows that the behaviour pattern which is usual for 'natural conditions' is upset by illumination from below. It also suggests that the winkles which settle on vertical surfaces begin their feeding excursions partially as a negative phototactic response, as probably do also those which have settled on sloping rock surfaces. Other observations on the behaviour of winkles in diffuse light confirm the view that under these conditions they crawl across the light rays. For example, it is the almost invariable rule that winkles placed in a glass aquarium some distance from a north window settle on east and west walls. That is, after a preliminary period of crawling, they ascend and settle on the walls which are not directly facing or away from the light. This behaviour is quite consistent with that observed for the distribution on the beach groynes, on which it is noticed that the vast majority of the animals are clustered against the junction of the vertical posts with the planking.

Summary of observations on the reactions of winkles to light

(1) Dark-adapted winkles from horizontal surfaces almost invariably behave positively phototactically but after some time of exposure to a light source they crawl horizontally across the beam and then, later still, behave negatively phototactically.

(2) Winkles from horizontal surfaces kept for some hours in diffuse light usually crawl transversely to a light beam.

(3) Winkles usually, but not always, disregard a second light source when this is switched on after they have orientated to the first source but sometimes they re-orientate towards the second light. They are thus able to behave telotactically to light.

(4) Winkles from vertical surfaces behave to light in a way which in several respects resembles the behaviour of those from horizontal surfaces. Thus, after being kept in diffuse light, they orientate and crawl across the light beam, and reverse the sign of their response when exposed to light after being dark-adapted. But there is an important difference, namely that most winkles from vertical surfaces are, when first tested, photonegative.

(5) The behaviour in the laboratory seems to be similar to that noticed on the shore, but suggests that winkles both from horizontal and vertical surfaces carry out feeding migrations which are influenced by light. Those from horizontal surfaces orientate *solely* by reactions to the direction of light, whilst those from vertical surfaces orientate to the direction of the light (which is always mainly from above) and also to gravity, the two sets of responses normally reinforcing each other.

DISCUSSION

The results of previous work on the behaviour of littorinids can now be considered, but, as will be apparent, the accounts of shore observations and the results of laboratory experiments on the reactions of winkles to light and to gravity are so conflicting that they give little help in the solution of the problem of the relation of these reactions to observed distribution of *Littorina littorea*. Indeed, the interpretation of these results is itself an interesting study, since it cannot be reasonably doubted that they are based on accurate, even if incomplete, observations. But the explanations offered in the present paper are at variance with previous accounts. To what, it may be asked, can these discrepancies be attributed? In part the answer is that previous workers failed to take due note of the immediate past history of the animals they studied, the exact situation from which the winkles were collected, the degree of dark-adaptation and so on. In part they are due to failure to make continuous observations on individual winkles, with the result that few authors noticed the regularly recurring reversal of the responses to light and gravity and none appreciated their significance in the mode of life of the animals. On the other hand, many, if not most, of the observations previously recorded can be confirmed, or partially confirmed, although receiving quite different explanations.

Bohn (1904*a*) stated that *Littorina rudis* has a natural rhythm of active and passive life, the whole cycle lasting 15 days, which is due to the varying heights reached by the tides over the lunar cycle. At spring tides the animals are active whilst at neaps the operculum closes and the animals are inactive. This is certainly not true for *L. littorea* and has not been confirmed for *L. rudis*. Bohn also stated that this rhythm is retained by *L. rudis* for several months in a laboratory, and he notes that at the time of spring tides the animals become more strongly positively geotactic and phototactic. Bohn (1904*b*, 1905*a*) states that on a horizontal surface an animal will crawl towards a light and then later reverse its direction, the time of reversal corresponding with the time at which in nature it would have experienced the maximum degree of dehydration on the shore. Again, referring to 'les littorines', Bohn (1904*c*) believed that on sloping surfaces winkles orientate along a resultant of the 'forces' due to negative phototaxis and negative geotaxis. Yet, when an animal is upside down on a vertical surface, negative phototaxis is said to be replaced by positive phototaxis. It can be said that these observations have not been confirmed for *L. littorea*, although, as has been seen, reversal of the sign of the response to light and gravity play a most important part in the feeding excursions. Later, Bohn (1905*b*) seemed to show that *L. littorea* is always negatively phototactic, but, as in previous papers, no details are given of his experimental procedure.

Mitsukuri (1901), working with the Japanese snail, *L. exigua*, stated that as a rule they are negatively phototactic and that, since the light is always more intense offshore(???), the animals are driven upshore away from the sea. The animals show a 'disinclination' to become submerged, as is shown in an aquarium by raising the water level. This he terms 'negative hydrotaxis'. But, as has been mentioned (p. 248), an exactly comparable behaviour has been seen for *L. littorea*, for which it is suggested that this is a result of the animals becoming quiescent after the termination of their feeding excursions. Mitsukuri believed that some winkles get left above the waterline but when re-wetted they become positively phototactic and move down the shore. Mitsukuri, it should be noted, paid no attention to gravity responses. Morse (1910) believed that his observations showed that *L. littorea* is negatively phototactic during the daytime in June but becomes positively phototactic as night approached. But, after 18 June, the numbers of winkles behaving in a positively phototactic manner predominated even during daytime, whilst the period of transition of the light responses corresponded with the time of change from spring to neap tides during which time winkles in a natural state on the rocks were exhibiting a corresponding change in phototaxis because the tide did not reach them. Morse therefore believed that he had confirmed the observations of Mitsukuri who stated that winkles, when desiccated, become positively phototactic and when submerged negatively phototactic. It is to be noted that Morse overlooked any possible reactions to gravity and that he gave no description of his experimental procedure. Haseman (1911), working on *L. littorea* at Woods Hole, was unable to confirm Bohn's results on tidal rhythmicity. He observed that winkles placed on horizontal surfaces do not show rhythmical movements corresponding with the rise and fall of the tides, but that snails located on more or less vertical surfaces follow the surface film of the water as it is raised or lowered. I have not been able to confirm this, but as has been stated (p. 248) winkles after a quiescent period crawl down and then up again to settle at or near the surface of the water. One curious observation which may be mentioned is that the winkles with which Haseman worked stopped opposite olive-green sectors in an aquarium in preference to sectors coloured yellow or brown. Haseman explained the zonation of winkles at Woods Hole along the following lines. Submerged animals crawl at random but do not crawl on dry surfaces and are not found above tidemarks. As the tide rises some, by mere chance, crawl above the waterline but the dryness and unevenness of the surface slows them down so that the tide overtakes them and they become submerged. When the tide falls, a few may get left behind and cling to the rocks.

Kanda (1916) also made studies on *L. littorea* and pointed out that earlier workers had omitted to take into account reactions to gravity. When winkles were allowed to crawl on a glass plate submerged in water he found that the steeper the angle of the plate, the greater was the number of negatively geotactic animals, but even on slopes as gentle as 11 degrees, 55% of the animals were negatively geotactic whereas, at angles of 90 degrees, 100% were negatively geotactic. Similar results were got with winkles crawling on a plate in air, but when a ground-glass plate was used 15% of the specimens behaved positively geotactically even at slopes of 90 degrees. Kanda interpreted this to mean that winkles tend to become positive when dry and points out that this 'would evidently serve as a protective reaction when they are left by the retreating tide'. When Kanda's results are examined in detail the striking thing is the irregularity of the gravity responses. For example, when a wooden plate was substituted for a glass one, in air, at an angle of 90 degrees, 52% of the winkles were positively geotactic, 16% were negative whilst 15% crawled horizontally. The rest fell off the plate.

The result of this experiment, which was made in darkness, was interpreted to mean that *positive* geotaxis predominated, but since Kanda failed to notice the reversal of the sign of the response to gravity the difference in the result from that of previous experiments may well have been due to the circumstance that he was observing downward, horizontal and upward parts of U-shaped tracks now known to be typical of winkles on vertical or steeply sloping surfaces at Whitstable. In nature Kanda thought that 'the limitation of upward movement is due chiefly to the exhaustion of moisture carried by the foot from the sea. They seldom, if ever, crawl on dry rocks much higher than high tide mark....They seem to stop crawling when the moisture which they carry themselves is exhausted.' When tested under similar conditions, except that the apparatus was half-filled with water with the plate at 90 degrees, no winkles were positively geotactic but twelve out of 150 crawled horizontally on reaching the surface film. In direct sunlight with the light parallel to the plate the winkles which were 'negatively heliotropic' showed a variety of reactions: 10% crawled diagonally, 16% went horizontally whilst 74% crawled downwards, so that both light and gravity seem to affect the direction of travel. Discussing these results and those of previous workers, Kanda states that *L. littorea* is, as believed by Bohn (1905*a*, *b*), 'negatively phototropic'. Indeed, Kanda places great emphasis on this point which, he says, is 'the unmistakable reaction of the animal to light'. Winkles (he says) do not, as supposed by Morse, crawl upwards because of 'positive heliotropism' but on account of 'negative geotropism', and in spite of 'negative heliotropism', and he further believes that Morse's view that as night approaches winkles become increasingly positively phototactic is incorrect. Haseman's results also come in for criticism by Kanda who (quite correctly) says that they are not clearly set out and that no account was taken of possible reactions to the sun's rays. Here again, it may be remarked, Kanda failed to notice the regular reversal of responses to light.

Gowanloch & Hayes (1926) were primarily concerned with the behavioural features affecting the zonation of *L. littorea* on a rocky shore. They state that winkles are never found on areas of fine sand or mud but are typically inhabitants of exposed rock surfaces, from which it is obvious that their remarks do not apply to the many situations other than rocks where winkles flourish. They believed that, although not expressible in a quantitative way, their observations on marked winkles showed that the animals returned to a particular tidal level when displaced. No evidence for this has been found in the present investigation. Their results also seemed to show that winkles varied in the degree of their response to light and to gravity. Those collected from high up on the shore are more strongly positively phototactic than those from lower down and desiccation steadily increases the degree of 'negative geotropism'. Later (1929) Hayes presented evidence for the view that increasing the time during which winkles are desiccated decreases the degree of 'negative geotropism' and that, conversely, prolonging the time of immersion increases 'negative geotropism'. Colman (1933), in a valuable discussion of the factors underlying zonation, pointed out that 'the combined effect of these reactions is to make it difficult for each species to get away from its normal habitat'.

This may well be true, but, as has been mentioned (p. 251), these differences in behaviour were probably caused not by differences in shore level as such but by variations in the steepness of beach slope, the lower levels being less steep than the upper ones.

On the flat type of shore, such as the 'wattens' and 'waddens' of the north German and Danish coasts, Schwarz (1932) stated that gravity responses play little or no part in the orientation of the winkles found there. When the sun is low and the light is directional, winkles behave positively phototactically when uncovered by the tide. Thus, they move from east to south in the morning and from south to west in the evening. On dull days and when the sun is high in the sky they move in a random manner. Ankel (1936) was unable to confirm what he terms 'this remarkable finding', yet had Schwarz noticed the reversal of the direction of crawling, his observations would have been substantially correct.

Reference may be made to papers dealing with the behaviour of species of *Littorina* other than *littorea*. Fraenkel (1927) carried out a thorough investigation of the reactions of *L. neritoides* to light and to gravity and convincingly showed that when submerged the animals are photonegative except when upside down (as when crawling on the ceiling of a rock crevice) when they are photopositive. Moreover, they are usually geonegative and so tend to crawl up rock faces. These reactions serve to explain why *L. neritoides* moves to the upper shore and splash zone. If when moving under water an animal encounters a crevice its negatively phototactic behaviour takes it inwards along the floor; its negative geotaxis causes it to climb the end wall and brings it to an upside-down position when it behaves positively phototactically and crawls along the ceiling out of the crevice. Yet when out of water, as when its movements have brought it above the tidal level, upward crawling is continued and if it enters a crevice as a result of negative phototaxis, it stays there, for the sign of this response does not reverse until the animal becomes submerged. This analysis goes a long way to explain the observed distribution of *L. neritoides*, which commonly inhabits crevices near and even above H.W.S., but that it is incomplete is shown by the observations (Lebour, 1935; Lysaght, 1941) that the animals are sometimes found in numbers in situations where they are permanently submerged. Moreover, at whatever tidal level it is found, *L. neritoides* must, presumably, leave its crevice on occasions in order to feed. It must be supposed, therefore, that the behaviour of this winkle is less stereotyped than Fraenkel's account would indicate.

A recent paper by Barkman (1955) deals with the reactions of *L. obtusata*. He states that this species is negatively geotactic and that this agrees with the results of all previous observers of littorinids except Mitsukuri (1901) and Morse (1910). *L. obtusata* is also invariably negatively phototactic. Barkman could detect no tidal rhythms and found that in a tank with a sloping floor and with an arrangement for simulating a rising and falling tide with an oscillation of 33 cm, the animals came to rest at about 6 cm above the waterline. This he explained by assuming that winkles have a tendency to crawl upwards until halted by desiccation. Similar movements occur on the shore, it is said.

The view of the locomotory pattern of behaviour of the common winkle, *L. littorea*, presented in the present paper, reduces the responses to a rather simple but reversible reaction to the direction of the incident light, which can be reinforced by gravity responses. All the winkles seem to spend most of the time in which they are submerged by the tide or exposed to the air, settled on places which offer good holding ground. But when stimulated, as by increased wave action during a rising or falling tide, they are activated and make feeding excursions of limited duration out from and back to their approximate place of settlement, thus tracing out roughly U-shaped tracks on the substratum, whether it be the sand or the vertical face of a groyne. The physiological explanation for the reversal of the sign of responses is not known, but, since it occurs even when the external environment is maintained constant, as under laboratory conditions, it must depend on some internal mechanism, and, following Pittendrich (1956), this may be termed a biological clock but one which does not fall readily into any one of the categories he specifies.

On very flat shores, such as occur in the Whitstable area, only two types of situation are (apart from very occasional small boulders) available for winkles to settle on, viz. the practically flat beach and the vertical surfaces of groynes, but on rocky shores winkles are presented with surfaces which have a great variety of slopes. It is highly likely, therefore (and preliminary observations at Plymouth reinforce this view), that the behaviour of winkles on rocky shores is more complex than that on flat shores in the sense that their feeding excursions are always determined in part by the direction of the light and in part by gravity, the two stimuli varying in importance according to the position of settlement, whereas, as has been seen, dwellers on flat surfaces orientate solely in response to light and those on vertical surfaces mainly in response to gravity.

The ecological importance of this type of behaviour would seem to be that a mainly sedentary animal is able to maintain its station of the shore in a position of security, but also, by reacting to environmental clues, to move out and return to a place of settlement, feeding meanwhile. The kind of behaviour exemplified by winkles finds a more precise expression in the movements of other intertidal animals, of which the common limpet, *Patella vulgata*, may be singled out for comment. Here the feeding excursions, which have repeatedly been described, end with a return to an exact spot or scar on the rock from which the animal began its crawl. Limpets can be truly said to return to a home. But it cannot for a moment be supposed that this type of behaviour, although outstanding in its precision, is unique. Rather it would seem proper to regard it as the extreme of a series at one end of which are intertidal animals that wander 'at random', and at the other those which regularly leave and return to a strictly localized spot. Winkles seem to occupy an intermediate position in this series and are, perhaps, representative of the vast majority of shore animals, although this is not to say that the environmental clues selected and the reactions to them are identical in all instances. Some, indeed, are unexpectedly complicated. For example, Papi & Pardi (1953) and Pardi & Papi (1953) showed that *Talitrus saltator* makes excursions after emerging from the sand and orientates by means of a light-compass reaction, moving up and down the beach at right angles to the shoreline, no matter what the time of day, and so returning to the zone of moist sand. This implies that in some way the animals make allowance for time in fixing their angle of orientation to the sun.

There can be little doubt that, as stressed by Ewer (1956), the distribution of animals on the shore is a dynamic process initiated by larval behaviour and in some instances maintained by the behaviour of the adults.

Winkles are structurally adapted in several obvious ways to life in the intertidal zone, to which, indeed, they are virtually restricted, few if any occurring below E.L.W.S. even when the nature of the sea floor is suitable and food is abundant. Bearing in mind that *L. littorea* can withstand prolonged immersion—for more than 50 days (Hayes, 1929)—the reasons for a fairly sharply defined lower limit to the zonation are obscure, whereas, other considerations apart, inability to withstand continued exposure and lack of suitable food suffice to account for the upper limit at approximately H.W.N. The results of the present investigation suggest that the lower limit of zonation may be that at which there is too little mechanical disturbance and too low a level of light intensity to stimulate winkles to embark on the feeding excursions, so that any which by chance settle below tidemarks are by their behaviour inhibited from feeding and so fail to become established.

SUMMARY

Winkles dwelling on horizontal surfaces orientate by means of a light-compass reaction. As the tide recedes, most of the tracks in the sand are towards the sun but after a time each animal reverses its direction of crawling, so tracing out a roughly U-shaped path which leads it back, approximately, to its starting-point.

When kept in an aquarium winkles tend to settle on the sides above the waterline and to become inactive. When stimulated, as by immersion, they crawl downwards, then horizontally and then upwards before settling above the waterline.

Winkles collected from horizontal surfaces at first show no tendency to climb vertical surfaces in an aquarium. After a period which varies from a few hours up to 10 days they do climb the sides of the tank. Animals collected from vertical surfaces climb the sides of the aquarium at their first opportunity and then settle. It can be concluded that winkles have an inherent tendency to

climb and settle in the head-up position on vertical or steeply sloping surfaces but that if this opportunity is denied them they become habituated to horizontal surfaces.

The minimum angle to which winkles can react by gravitational responses is of the order of 10–20 degrees.

In an aquarium starved winkles prolong their excursions below water when presented with suitable food but fully fed animals respond less strongly to the stimulus of immersion.

Dark-adapted winkles from horizontal surfaces are at first positively phototactic, but after a time varying from 15 to 20 min, they become negatively phototactic. Tested with the 'two-light' experiment most animals disregard the second light (telotaxis).

Dark-adapted winkles from vertical surfaces are at first negatively phototactic and then from periods of 5 min and upwards photopositive. This behaviour agrees with that seen on the shore, where their feeding migrations are orientated responses to gravity but are reinforced by reactions to light.

The relations of the responses of winkles to light and to gravity to the observed pattern of zonation are discussed.

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THE BREEDING OF *ARENICOLA ECAUDATA* JOHNSTON AND *A. BRANCHIALIS* AUD. & EDW. AT PLYMOUTH

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(Text-figs. 1–9)

The two 'tail-less' lugworms, *Arenicola ecaudata* Johnston and *A. branchialis* Audouin and Milne-Edwards (= *A. grubii* Claparède), live in gravel and under stones, unlike the 'tailed' species which are found mainly in sandy beaches. Both are local in distribution compared with the wide-spread caudate species. *A. ecaudata* is known to occur from Iceland to northern Spain, and thus has a rather more boreal distribution than *A. branchialis* which ranges from the west coast of Scotland to Morocco, the Mediterranean and the Black Sea. (Ashworth, 1912; McIntosh, 1915; Fauvel, 1927; Rioja, 1935; Wesenberg-Lund, 1951.) Neither species is known from the western side of the Atlantic.

Since the two species can often occur side by side and it appeared that they were adapted, not for life in different habitats, but for different temperature régimes, it was suggested to us by Prof. G. P. Wells, F.R.S., that an investigation of their breeding seasons at Plymouth might help in understanding their distribution and specific separation. It happened that we had already made some preliminary investigations in the Isle of Man, and we were therefore pleased to adopt Prof. Wells's suggestion.

Mature specimens of both species have been noted by previous workers. Ashworth (1912) found mature gametes in *A. ecaudata* at Port Erin in April and at Plymouth in August. Fauvel (1899) recorded mature worms at Cherbourg from March to October, while Hentschel (1930) believed spawning occurred at Plymouth in February–March and August–September. It thus seemed possible that *A. ecaudata* spawned during the warmer half of the year. *A. branchialis*, on the other hand, was considered to be mature in September–October in Britain (Ashworth, 1912) and in winter at Naples (Lo Bianco, 1909). Samples of both species examined by ourselves at Port Erin showed no such difference in periods of maturity. Apparently mature gametes of *A. ecaudata* were found during the winter and of *A. branchialis* in the spring. It was therefore clear that it might be necessary to sample both species for more than a year to determine the breeding period with certainty.

The work at Plymouth has confirmed that both species may be breeding at the same time, but has shown significant differences in the breeding cycle. The results help to explain certain aspects of the behaviour, development and systematics of the species.

We are indebted to Prof. G. P. Wells for ideas on which the work was based, and for encouragement and advice. One of us was in receipt of a D.S.I.R. senior research award during part of the work.

MATERIAL

A. ecaudata and *A. branchialis* are fairly common on most shores around Plymouth, in gravel or sandy gravel under stones. According to Fauvel (1927) the distribution of these species on the shore, in France, is as follows: *A. ecaudata* is found 'à un niveau assez bas', while *A. branchialis* lives 'jusqu'à un niveau découvrant à toutes les marées. A un niveau plus bas elle est souvent mélangée à *A. ecaudata*.'

Some of our samples were collected at Drake's Island, in Plymouth Sound, by Mr A. C. Briggs, but most of the material used was collected by the authors at Wembury Beach. Here a small sandy beach separates two masses of rock, Church Reef and West Reef, each with numerous gullies filled with gravel and boulders. The two species of *Arenicola* occur together in these gullies, from about mid-tide level to extreme low water mark. Between October 1955 and August 1956 we kept separate our samples from different tide levels. On Church Reef 201 worms were collected between L.W.S. and L.W.N.; of these 177 were *ecaudata* and 24 *branchialis*. Between L.W.N. and M.T.L. the total was 210, of which only 97 were *ecaudata*. The L.W.S. to L.W.N. level of the West Reef produced a total of 244 worms, including 197 *ecaudata*. These results agree in the main with Fauvel, except that *ecaudata* was found mixed with *branchialis* at all levels, not only towards low water.

Although the figures suggest that there were about equal numbers of the two species between L.W.N. and M.T.L., *branchialis* retreats rapidly into its burrow and more were present in the gravel than were collected in our samples. Thus *branchialis* may form rather more than 50% of the population above L.W.N. Above M.T.L. and below M.L.W.S. we found a few specimens of both species, but in too small a number to determine the real proportions.

Apart from this difference in abundance with tide level, we noted that at all inhabited levels *A. branchialis* was the commoner in patches of sandy gravel which were more exposed to wave action, while *A. ecaudata* was often found alone in sheltered hollows where the gravel contained much sea-weed debris and decaying organic matter. However, both species have been found at wave beaten places such as Rame Head, where they occur side by side in gullies of coarse gravel; both species can also be found in places where the 'black layer' (Bruce, 1928) lies close to the surface. There thus seems to be no constant correlation with type of deposit or organic content in either species. When both species are considered together and compared with the only other species of

Arenicola present in the British Isles (*A. marina* L.) there is a clear separation of habitats. At Plymouth and the Isle of Man we have found *A. marina* together with either of the other species only twice in samples totalling over 2000 worms.

METHODS

The investigations began in May 1954 and sampling was continued at irregular intervals through 1954 and 1955. Experience gained from these samples enabled a more regular series of samples, from November 1955 to January 1957, to be sexed and classified with greater accuracy. The samples were collected during spring tides, at monthly or fortnightly intervals during this period.

We planned to examine between twelve and twenty specimens of each species at a time, but owing to the irregular nature of the habitat, and the variable proportions of the species, some samples contained fewer than twelve specimens of each. We have omitted from our results samples of less than ten *A. ecaudata* and less than seven *A. branchialis*. The latter was always more difficult to find and it was necessary to search the same areas each time. However, there was no marked decline in abundance, and it is assumed that the vacant niches were filled up by migration of worms from surrounding areas.

In the laboratory the worms were first cleaned of gravel and mucus and sorted into species. For this purpose we relied on the differences in the number of abranchiate setigerous segments (Fauvel, 1927).

In the second series of samples the size of each worm was measured as volume (to the nearest 0·1 ml.) of sea water displaced, but this was not done before November 1955.

Measurement of volume was followed by an examination of a drop of coelomic fluid from each worm.

In *A. ecaudata* most of the development of the gametes takes place in the gonads and the sex-products do not appear in the coelomic fluid until they are nearly mature. Thus, in this species, if the coelomic fluid contained no gametes the gonads were examined, and one of them removed for measurement of the oocytes. Our ability to sex immature and spent specimens of *A. ecaudata* increased during the survey, and this probably accounts for the absence of worms of unknown sex from the 1956 samples.

In *A. branchialis* the gonads are very small (see Fauvel, 1927; Downing, 1909, 1911) and the gametes undergo most of their development in the coelom. It was therefore a simple matter to sex and classify this species by examining some of the coelomic fluid under the microscope.

Males and females were grouped into the developmental stages shown in Table 1, and the largest oocytes were measured with an eye-piece micrometer. The divisions of this micrometer (17 μ with $\frac{2}{3}$ in. objective) were used to define the eleven oocyte stages in this species and the ten stages in *A. ecaudata* (see Table 2). In both species the oocytes are slightly oval in shape and the greatest diameter was measured.

TABLE 1. DEFINITIONS OF BREEDING STAGES

		Arenicola ecaudata
Male	Empty (E)	Sex determinable from shape of gonads, no sex products visible in gonads or coelom
	Developing (D)	Sex products in gonads, none in coelom
	Mature (M)	Coelom full of tailed sperm plates or free spermatozoa
	Spent (S)	A few sperm plates or spermatozoa in coelom, gonads empty
Female	Empty (E)	As male
	Developing	
	(1) in gonads (Dg)	Oocytes developing inside gonads, none in coelom
	(2) in coelom (Dc)	Coelom contains oocytes, of which less than 90 % have reached 0·17 mm in diameter
	Mature (M)	Coelom full of oocytes, of which at least 90 % are 0·17 mm or more in diameter
	Spent (S)	Gonads still large but empty of oocytes, occasionally a few large oocytes in the coelom
Sex unknown	Empty (E)	Gonads very small or absent, no sex products visible
		Arenicola branchialis
Male	Developing (D)	Coelom contains sperm morulae and/or sperm discs, of which less than 90 % are tailed
	Mature (M)	Coelom contains sperm discs of which at least 90 % are tailed
	Spent (S)	A few tailed discs or spermatozoa in the coelom
Female	Developing (D)	Coelom contains oocytes, of which less than 90 % have reached 0·17 mm in diameter
	Mature (M)	Coelom full of oocytes, of which at least 90 % have reached 0·17 mm or more in diameter
	Spent (S)	Coelom contains a few large oocytes, with often a few very small
Sex unknown	Empty (E)	No sex products discernible

TABLE 2. DEFINITION OF OOCYTE SIZE-GROUPS

Group	Diameter of largest oocyte present	Group	Diameter of largest oocyte present
0	Ovary distinct but no oocytes visible	6	0·086–0·102 mm
1	Up to 0·017 mm	7	0·103–0·119 mm
2	0·018–0·034 mm	8	0·120–0·136 mm
3	0·035–0·051 mm	9	0·137–0·153 mm
4	0·052–0·068 mm	10	0·154–0·170 mm
5	0·069–0·085 mm	11	0·171–0·187 mm

ARENICOLA BRANCHIALIS

The sex of *A. branchialis* could not be determined from the gonads, so that when there were no sex products in the coelomic fluid the specimen was classified as 'of unknown sex'. The developing gametes, whether male or female, were shed from the gonads at an early stage in their development, and appeared in the coelomic fluid a few at a time over a period of months; gametes at all stages of development might be found in the coelom of one worm. After the production of gametes ceased maturation continued until all were full sized.

The development of sperm discs from balls of spermatogonia has been described by Downing (1911) for several species of *Arenicola*, including *branchialis*. In the females the oocytes were shed from the ovaries at a diameter of 17 μ or even less. They grew steadily in size to a diameter of 0·17–0·187 mm when mature. At a diameter of about 0·12 mm a thick vitelline membrane was formed. The mature oocytes have been described and figured by Ashworth (1912). After spawning, a few mature oocytes remained in the coelom of the female, often being mixed with the first small oocytes produced by the gonads as the cycle began again.

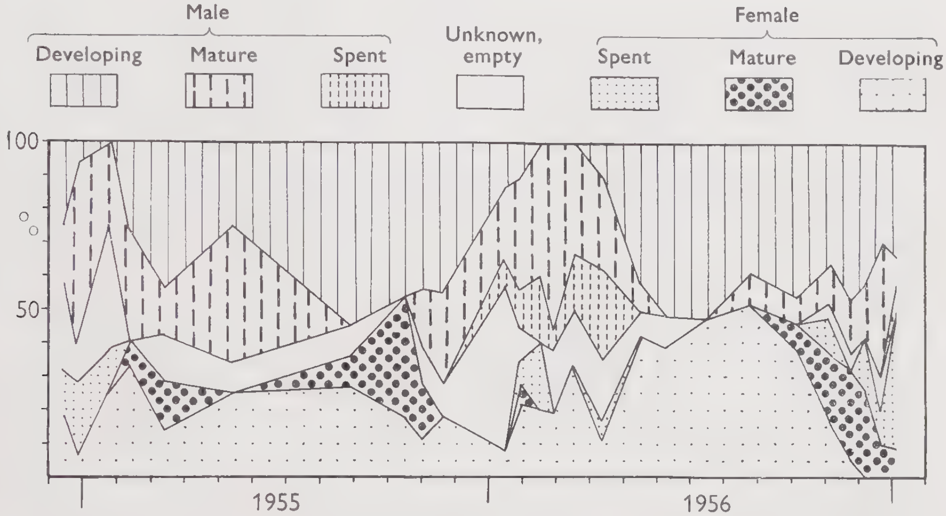

Fig. 1. *Arenicola branchialis*. Percentages of breeding stages (see Table 1) in samples from Wembury, December 1954 to January 1957.

Annual cycle

The results of 2 years' sampling are shown in Fig. 1, the developmental stages being those of Table 1. Samples were few in 1955 and included some small specimens; from 15 November 1955 specimens 1 ml. or under in volume have been omitted from the figure, since we believe that young specimens may develop at a different rate, and there seems to be a fairly steady recruitment of young specimens into the breeding population throughout the year.

The general pattern of development and spawning seems to have been the same in 1955 and 1956. The gametes developed throughout the spring, summer and autumn, and spawning took place over a period of 4–6 months centring on the winter. The 1954–55 spawning did not finish before the end of January, and may have gone on longer. Spent specimens were not found after the end of January, but mature females and males remained in the population until at least March and May, respectively. Development began again soon after spawning, while many individuals still had a few residual gametes in the coelom. Development of the females continued until the end of October 1955, when more than half of them were mature, though apparently all the males were still developing. Fig. 2 shows the development of the oocytes of the female population throughout 1955 and 1956. Clearly, growth of oocytes continues slowly and steadily up to midsummer; thereafter, most of the population is mature for 3 or 4 months before spawning.

Spawning

In December 1954 the occurrence of spent females and some empty specimens indicated that spawning had already begun. During the following winter, 1955–56, samples were not taken in December but spawning apparently began about the same time, for spent specimens were found in January. Spawning may indeed have begun earlier, if the decrease in the proportion of females in the samples in October and November can be attributed to post-spawning mortality. Spent males were found in the samples up to April 1956, although the majority of females must have spawned before the end of February.

From the remaining samples it seems clear that the 1956–57 spawning began between early October and early November and was still in progress in mid-January when sampling ceased. The females again showed a drop in number that could be attributed to post-spawning mortality. In both spawning periods it seems that spawning began first in the females, while from the spring samples of 1955 and 1956 the males seem to have finished spawning later than the females. Thus, although the whole spawning period lasted 6 months, the two sexes apparently spawned together for only 4 months.

In *A. branchialis*, as in other species of *Arenicola*, spawning takes place through the nephridia and not through a breakdown of the body wall. Nevertheless, the sudden variations in the ratio of the sexes during the spawning season suggest that a number of worms of both sexes die after spawning, as in *A. marina* (Newell, 1948). We have not had spawning specimens of both sexes in the same sample, and have not seen naturally fertilized eggs, nor have any of our artificial fertilizations been successful. The larva remains unknown, but the post-larva has been described by Ashworth (1912); his specimens were collected in Ireland, among *Laminaria* roots, by Southern who also described them (1914). Post-larvae have not been recorded from the Plymouth area. The smallest specimens found by us among the gravel with the adults were 2–3 cm long and had all segments and branchiae.

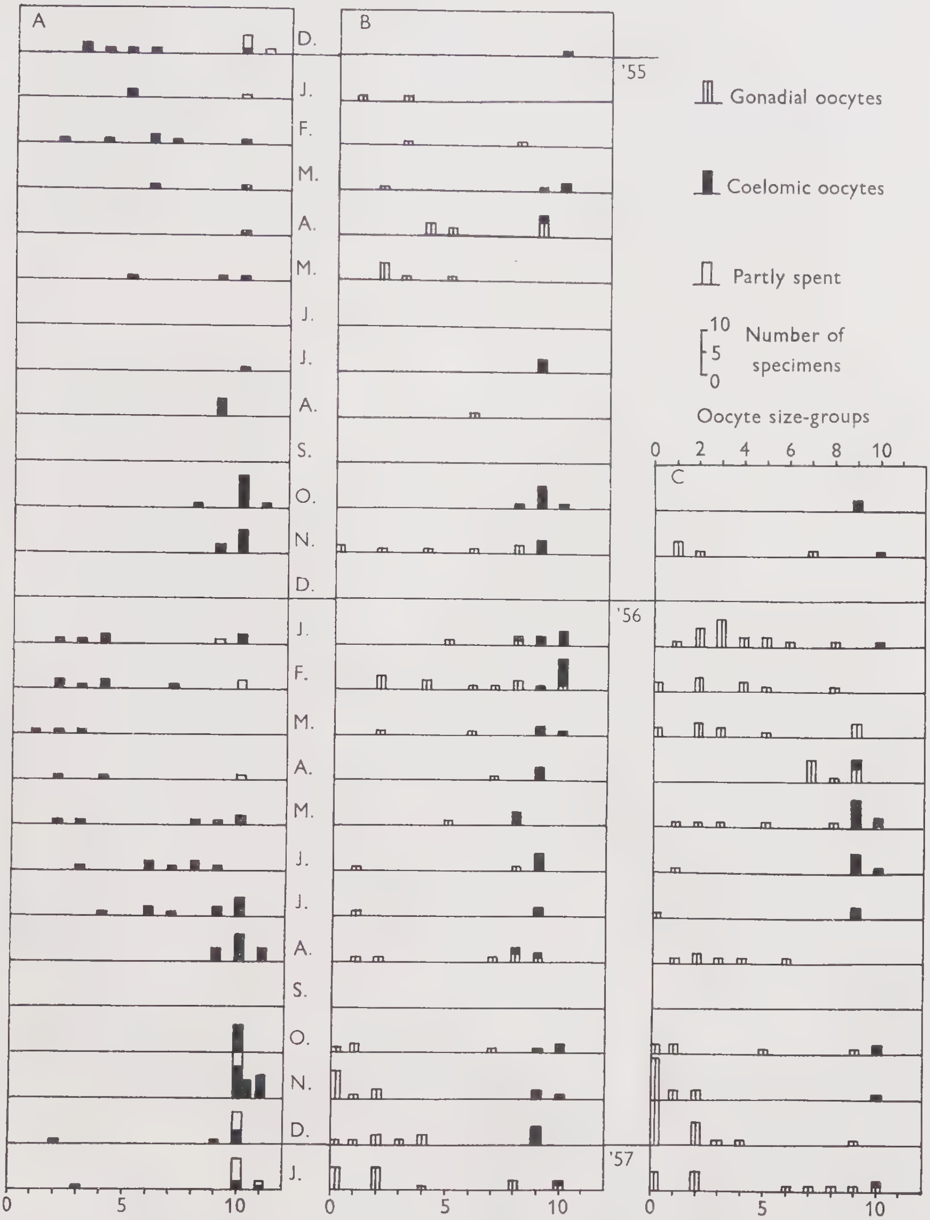

Fig. 2. Oocyte sizes. Number of worms containing oocytes of each size group (see Table 2), each month. A, *Arenicola branchialis*, Wembury; B, *A. ecaudata*, Church Reef, Wembury; C, *A. ecaudata*, West Reef, Wembury.

Parasites

The internal sporozoan parasites frequent in *Arenicola ecaudata* (see p. 277) are apparently absent from *A. branchialis*, but this species does bear on its branchiae a peritrichous ciliate, *Rhabdostyla arenicolae* Fabre-Domergue, already known from *Arenicola marina* (Cuénot, 1891). At Plymouth we have found *Rhabdostyla* on both *Arenicola marina* and *A. branchialis*, but surprisingly not on *A. ecaudata*. The ciliate does not seem to have any adverse affect on its host and must be regarded as epizooic or commensal.

ARENICOLA ECAUDATA

The sex of *A. ecaudata* could nearly always be determined by examination of the gonads. Those of the male are sac-like and only slightly lobed, while those of the female are divided into a large number of long narrow lobes (Ashworth, 1912). Even when empty of gametes or very small the gonads of the two sexes are different in shape.

In the male the first sign of development is the appearance of balls of spermatogonia in the gonads. These balls flatten into discs and develop tails before being shed into the coelom (Downing, 1911). The tailed discs remain in the coelom for some time; occasional worms were taken in which the spermatozoa were free, and some of these specimens spawned when handled. We do not know whether the discs always break up into spermatozoa before spawning.

In the female the oocytes were first visible in the ovaries at a diameter of about 8–17 μ. All were about the same size and seemed to develop at the same rate, while the gonad grew in size to accommodate them. The full ovaries usually filled the anterior part of the coelom. The oocytes developed a thick vitelline membrane at the same size as do those of *A. branchialis* (about 0·12 mm). They were sometimes shed into the coelom at this stage, but were usually retained until they had reached 0·137–0·15 mm. Once in the coelomic fluid they continued to grow in size until they were about 0·17 mm in diameter (stage 10).

In the male a few tailed discs or free spermatozoa remained in the coelom after spawning. In the female the coelom was completely empty of oocytes after spawning; the gonads of recently spent worms could easily be distinguished from early development stages by their large size and flabby condition.

Annual cycle

The results of 2 years' sampling of the Church Reef population are shown in Fig. 3, together with 1 year's results from the West Reef and Drake's Island populations. The two Wembury populations are shown separately, unlike those of *A. branchialis*, not only because the samples were sufficiently large, but because there seems to be some difference between them. The small specimens have been omitted from the figure, on and after 15 November 1955, as with *A. branchialis*. Before this date the classification 'sex unknown' probably included small undeveloped worms as well as spent and empty adults.

At all three sites, Church Reef, West Reef and Drake's Island, mature males of *A. ecaudata* were present practically all the year round. It is difficult to determine any definite cycle, although the least proportion of such males was found in late winter, the greatest in summer. This trend is best shown by the West Reef samples. From the minimum in January and February 1956,

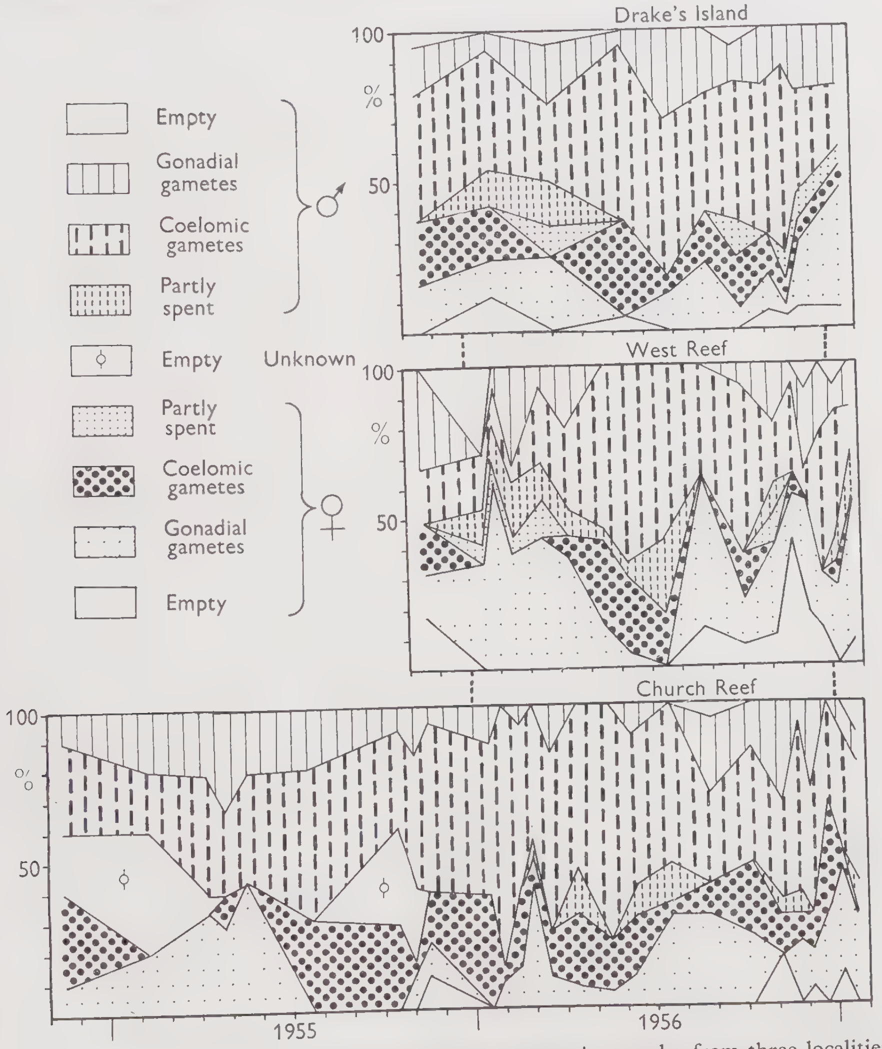

Fig. 3. *Arenicola ecaudata*. Percentages of breeding stages in samples from three localities.

when most of the males were either spent or developing gametes in the gonads, the number with coelomic gametes increased steadily to May, when over half the samples consisted of mature males. The proportion declined from June to August and at the same time the proportion of spent males increased. There was another increase in mature males up to September, after which the proportion declined, most of the males being in the gonad developing condition. A further peak of mature males occurred in December 1956, followed by another decline in January 1957. This suggests that several spawnings occurred during the year.

The annual cycle of the females is clearer, although apparently mature specimens may be found nearly all the year round. The high proportion of spent or gonadially developing specimens in January–March is gradually reduced as more and more specimens release their oocytes into the coelom, until by June or July most of the females appear mature. Thereafter, increases in the proportions of spent and developing females alternate with slight increases in the proportion of mature worms, indicating that several cycles of spawning and redevelopment occur until the next January or March. From measurements of oocyte sizes (see Fig. 2) two main periods of development and maturation are apparent, one beginning January–March and completed May–July, another beginning July–August and completed by December. No doubt lesser trends may be hidden by the smallness of the samples.

Spawning

The Church Reef population was sampled rather irregularly during 1955 and Fig. 3 does not show the spawning season very clearly. However, there seems to have been some spawning during the winter 1954–55, lasting until about February, while after this most of the females were redeveloping; the proportion of developing/mature males remained about the same as before. By July all the females and most of the males were again mature or almost so; the large number of empty worms the following October indicates that spawning had taken place. In November some more spawning and further redevelopment seems to have occurred. Samples were not collected in December, but early in January 1956 most of both sexes were mature again and the drop in the proportion of females probably indicates yet more spawning. During the winter of 1955–56 there seems to have been no cessation of spawning at all, and further spawning occurred, at least among the males, in March and April, although the proportion of mature females remained fairly steady and did not decline markedly until June. Spawning must have continued at intervals right through to the end of sampling in 1957.

In the West Reef population the spawning periods were a little more distinct, the main peaks occurring in January, March, July and November 1956, and January 1957. The Drake's Island population, however, was more comparable to that of Church Reef, in that spawning seemed continuous, although the main peaks were indicated by the presence of spent females in the samples.

It is worth noting that, in general, the deposit at the Church Reef and Drake's Island sites was richer in decaying sea-weeds and other organic matter than that at West Reef. The average size of the worms was greater at these two sites, and it is possible that the apparently continuous breeding activities there may have been due to the presence of more abundant food.

Effect of parasites

Two sporozoan parasites have been recorded from *Arenicola ecaudata* at Plymouth (Cunningham, 1907; Goodrich & Pixell-Goodrich, 1920), and both were found again during the present survey.

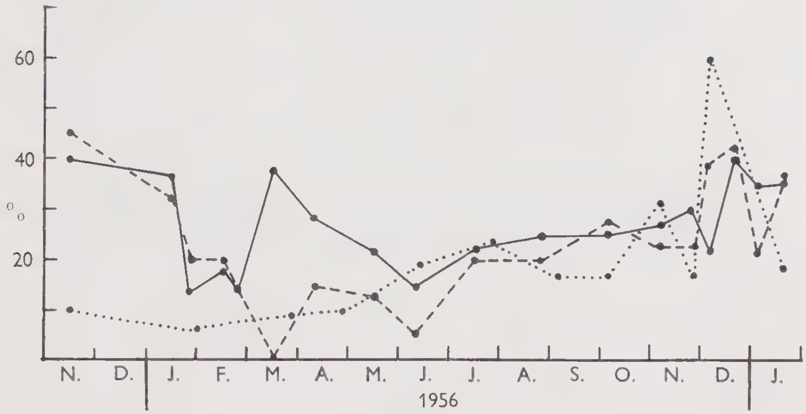

Fig. 4. Percentage *Arenicola ecaudata* containing cysts of *Gonospora arenicolae*, in each sample from three localities: ——, Church Reef; – – – –, West Reef; . . . ., Drake's Island.

The trophozoites of *Gonospora arenicolae* (Cunningham) live attached to the nephridia of both sexes of *Arenicola ecaudata*, dropping off into the coelomic fluid when mature and uniting in pairs or in groups to form gametocysts (Cunningham, 1907). These cysts are white spheres, about 1 mm in diameter, and obvious to the unaided eye. We did not examine the nephridia of every specimen but believe that most were infected with trophozoites (cf. Hentschel, 1930). We recorded the presence of gametocysts in the coelom and show in Fig. 4 their occurrence during 1956. There was a winter maximum in all three populations, coinciding more or less with the winter spawning. In the Church Reef population there was a second peak in March, just before the April spawning (see Fig. 3), but in the others there was a steady rise in rate of occurrence from February to November, with a rapid rise about December. Except in a few cases of extremely heavy infection, when the coelom was almost full of trophozoites and cysts, this parasite did not apparently affect the rate of development of the gametes, although, by using part of the available food supply, it must have reduced their numbers.

Gonospora minchini Goodrich & Pixell-Goodrich has trophozoites which attack the oocytes of *Arenicola ecaudata*, entering them sometime after they have been released from the gonads. The growing trophozoite enlarges the oocyte membrane enormously and destroys its contents. The trophozoites emerge from the membranes when full grown and unite in pairs or groups, as in *Gonospora arenicolae*. They form cysts which are, to us, indistinguishable from those of *G. arenicolae*. Since both parasites may be present in one worm, some of our records of *G. minchini* in the gametocyst stage may include *G. arenicolae* and vice versa.

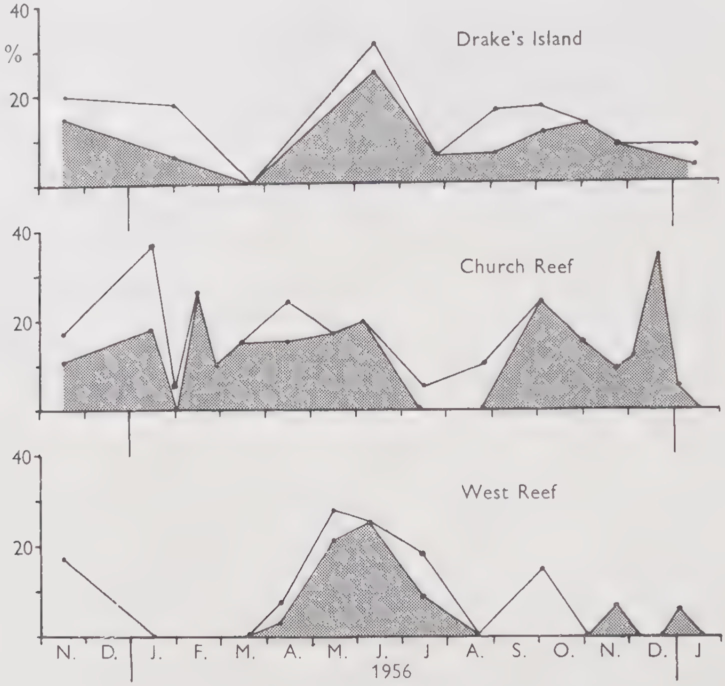

Fig. 5. Percentage *Arenicola ecaudata* containing coelomic oocytes in each sample from three localities. Percentage infected with *Gonospora minchini* stippled.

G. minchini apparently never attacks the oocytes while they are in the ovaries. We have not been able to discover whether there is some other site of infection in the worm during the development of the ovaries, but well over 50% of the worms with coelomic oocytes had this parasite in them. Fig. 5 shows the proportion of these females infected in each sample during 1956. Some of these were only lightly infected (1–10% of the oocytes attacked) but others had up to 50% of the oocytes destroyed. Though the presence of the parasite does not appear to affect the rate of development of the unattacked oocytes, it has a marked effect on the fecundity of the host.

Larval development

On two occasions (16 November 1954 and 2 November 1956) specimens of *Arenicola ecaudata* spawned in the laboratory and produced fertilized eggs, a few hours after the samples had been brought in from the shore. Hentschel (1930) noted that the worms spawned inside their transparent tubes, moving about as they poured out their genital products. We found the eggs embedded in these transparent tubes, which the worms always form when kept in the laboratory. The tubes, after being washed, were placed in finger bowls of filtered sea water and kept at a temperature of approximately 15° C.

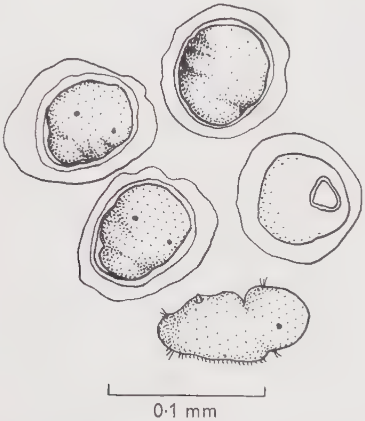

Fig. 6. *Arenicola ecaudata.* Group of larvae ready to hatch; with one already hatched and an empty egg membrane.

In the first case hatching was not observed, but the larvae were crawling about the dish on the eighth day. In the second case hatching took place on the eighth day, and before hatching the larvae were seen to twist about inside the egg membrane for some time. They finally emerged through a small hole (Fig. 6), crawled about the bottom of the dish and formed small transparent tubes; they were not seen to swim at any time.

The newly hatched larva (Figs. 6, 7A) had two circular bands of cilia with a ventral band between them (as in *A. marina* (Newell, 1948)), a pair of dorsal limbate setae and a pair of red eye-spots with dorsal lenses. During the next 2 or 3 days a second notoseta and an uncinate neuroseta appeared on each side; these were followed by the first limbate notosetae of the second setigerous segment (Fig. 7B). Table 3 shows the rate of development of the larvae. The second and third setigers developed in the same way as the first, but after this the first notoseta to appear on each side was uncinate and the limbate setae developed later (Table 4; Fig. 7C); this also occurs in other species of *Arenicola* (Ashworth, 1912).

The 1954 larvae developed only to the 4-setiger stage, but lived a further 14 days without any more growth. The 1956 larvae reached the 6-setiger stage and lived a further 40 days without further growth or development, although various algal cultures (kindly supplied by Dr M. Parke) were provided as food and were eaten by the larvae.

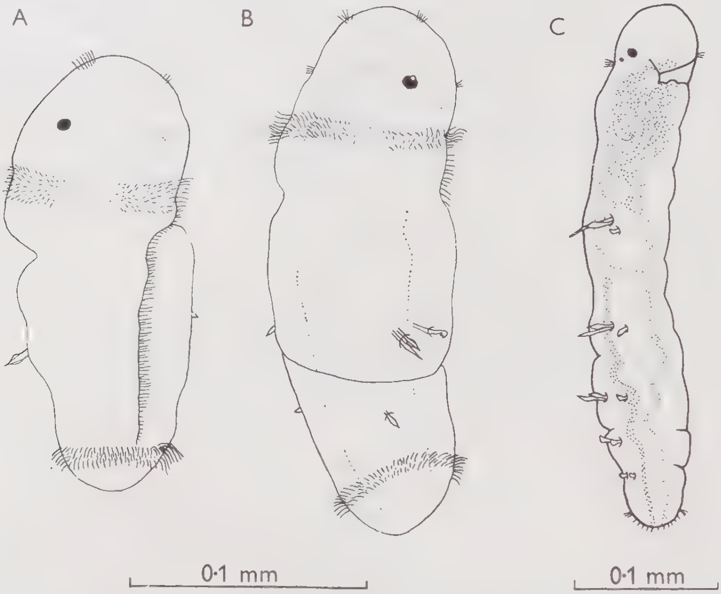

Fig. 7. *Arenicola ecaudata.* A, larva with one setigerous segment, showing ventral ciliated band; B, larva with two setigerous segments, side view; C, larva with five setigerous segments, side view (alimentary canal stippled). From camera lucida drawings of living larvae.

Behaviour of larvae

The larvae started feeding at the 4-setiger stage when the gut was completely formed and the mouth and anus open. The pharynx was ciliated and not yet eversible, though it was capable of some movement. The food taken was bacteria and small fragments of detritus, the remains being ejected as faecal pellets about $34 \times 17\mu$. A second pair of eyespots appeared, and, as the 5th setiger developed, yellow pigment was laid down in the prostomium, around the anus, and in the wall of the mid-gut. The proboscis grew larger and became eversible. By the 6-setiger stage it was well developed, and the larvae were feeding actively, picking up bacteria, algae and detritus from the bottom of the dish. The proboscis was everted in two stages, as shown in Fig. 8. By this time the larvae had developed a fairly definite pattern of behaviour, which consisted basically of backward and forward movements in the tube, which was about twice the length of the larva. When watched under the microscope, at 15–20° C, the larva moved back and forth once or twice a minute.

TABLE 3. RATE OF DEVELOPMENT OF *ARENICOLA ECAUDATA* LARVAE

Date of fertilization ...	16. xi. 54	2. xi. 56
Stage reached	Age in days	
Hatching (1-setiger)	8	8
2-setiger	11	10
4-setiger	17	20
5-setiger	—	28
6-setiger	—	34

TABLE 4. SETAE OF 6-SETIGER LARVA OF *ARENICOLA ECAUDATA*

Setigerous segment	Dorsal	Ventral
1st	2 limbate	1 uncinate
2nd	2 limbate	1 uncinate
3rd	2 limbate	1 uncinate
4th	1 uncinate 1 limbate	1 uncinate
5th	1 uncinate	1 uncinate
6th	1 uncinate	1 uncinate

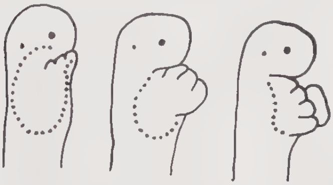

Fig. 8. *Arenicola ecaudata*. Two stages in eversion of the proboscis in a 6-setiger larva. Sketches of living larva.

During feeding the proboscis was extruded at the end of a forward movement, and there were up to twenty of these extrusions, at the rate of five to ten per minute, before the larva moved back. The next forward movement was followed by further extrusions of the proboscis, and so on for up to 15 min. Between periods of feeding activity there were periods of back and forth movement alone, lasting an hour or more, during which one or two faecal pellets were produced. Each of these was ejected at the end of a backward movement so that a heap of faecal pellets was built up a short distance behind each larva. The larvae would occasionally turn round in the tube and continue the same behaviour facing the other way.

Proboscis apparatus of the larva

The origin of the retractor sheath and gular membrane, or 'first diaphragm', of *Arenicola* has been the object of some speculation; in the adult the two structures are associated at their junction with the body wall and, while

Lillie (1905) considered that they originated separately, Wells (1952, 1954) suggested that they were derived from the splitting of a single septum. We have examined stained specimens of 1-, 2-, 4- and 5-setiger larvae of *A. ecaudata*, and find that in the last two of these the gular membrane and retractor sheath are already separate, although the membrane has not yet assumed its adult form (Fig. 9). The 1- and 2-setiger larvae are rendered opaque by the yolk they contain. Any splitting of the larval septum must take place before the 4-setiger stage; to show it clearly, horizontal sections of the larvae would probably be required.

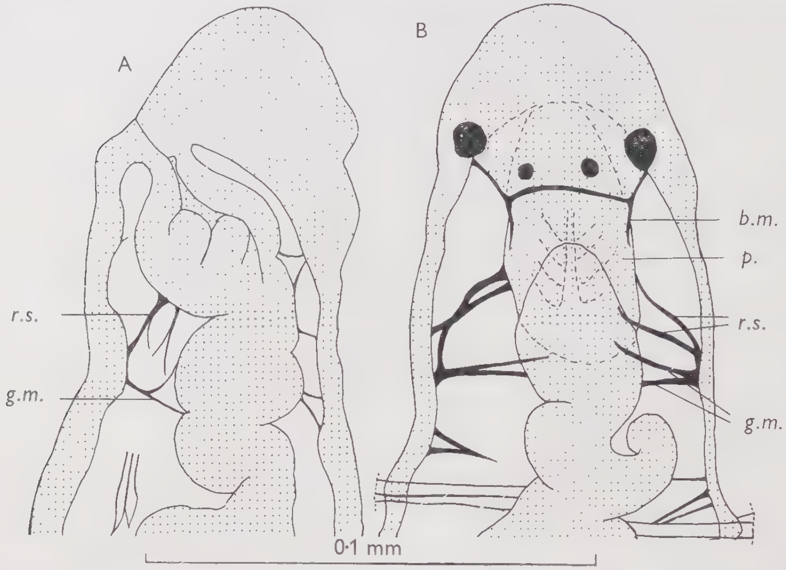

Fig. 9. *Arenicola ecaudata*. The appearance of the proboscis apparatus in stained whole larvae A, 4-setiger stage, from the side; B, 5-setiger stage, from above. Camera lucida drawings. *b.m.*, buccal membrane; *g.m.*, gular membrane; *r.s.*, retractor sheath; *p.*, pharynx.

Later development

The post-larval and juvenile stages of *A. ecaudata* can be found towards L.W.S. among small algae and in *Laminaria* holdfasts. Ashworth (1912) recorded them from Ireland in September and McIntosh (1915) found them in the Hebrides in August. We have found juveniles (less than 2 cm long) in the Isle of Man in the following months: January, March, April, May, July, August and September. We have found them at Plymouth in October but have not looked for them at other times of the year.

The smallest specimens found in the adult habitat at Plymouth were 2–3 cm in length and had a volume of 1 ml. or less. These occurred in our samples throughout the year.

COMPARISONS AND CONCLUSIONS

Having described the breeding cycles of *Arenicola ecaudata* and *A. branchialis*, we can compare them with one another and with those of other species. The absence of resting phases from these cycles distinguishes them both from that of *A. marina*, the only other species thoroughly investigated (Newell, 1948).

In spawning habits *A. ecaudata* resembles most closely the Japanese *A. claparedii* (Okuda, 1938), although the breeding cycles are not very similar. The larvae of *A. ecaudata* are slow to develop, in comparison with those of other species of *Arenicola*, but the larvae of all species are otherwise very much alike.

The breeding cycles of animals and plants are usually dependent in some way on annual fluctuations in external conditions, particularly temperature and food supply (Orton, 1920; Thorson, 1946; Qasim, 1956; Crisp & Southward, 1958). At Plymouth the same temperature régime is experienced by both *A. ecaudata* and *A. branchialis* and the same food supply is available to each. The amount of algal detritus on the shore varies considerably with the weather conditions, but even when it is at its lowest it is still abundant in the habitat of these species, though *branchialis* can apparently tolerate a lower organic content than can *ecaudata* (p. 268). It appears that the food supply should always be sufficient, and so we must look to temperature for an explanation of the difference between their breeding cycles.

According to Orton (1920), cosmopolitan species should have a wide range of temperature within which breeding can occur; cold-water species near their warmer limits should breed in the colder months of the year; warm-water species near their colder limits should breed in the warmer months of the year. Many organisms have been shown to conform to these conditions, but the two ecaudate species of *Arenicola* do not.

The temperature requirements of any species vary during its life history and during its breeding cycle. The geographical distribution may be closely linked to the temperature tolerance of the adult, but the breeding cycle is affected by different temperatures at each stage and this also may affect the distribution of a species. *A. branchialis* is nearing its northern limit at Plymouth and if it is limited by low temperature and not by any other factor then this may take effect in one or more of the following ways. A lower winter temperature than that normally experienced on the west coasts of the British Isles may kill the adults. Since the gonads are capable of initiating gamete production during the coldest part of the year at Plymouth it seems unlikely that gametes could not be produced, in the warmer months at least, further north; however, growth and maturation of the gametes are slow at Plymouth, taking up to 10 months. Further north maturation might not be completed in the shorter summer. Spawning takes place at Plymouth as the sea temperature is falling, and the larvae must develop during the coldest part of the year;

farther north lower temperatures on the shore might be lethal to the larvae, if not to the adults. The distribution of *branchialis* south of the Mediterranean area is presumably limited by the effect of high winter temperatures on the spawning or the larvae, but little is known of the breeding cycle there.

A. ecaudata is nearing its southern limit at Plymouth but it extends further north than *branchialis*. Since gametes can be produced and matured all the year round at Plymouth, these processes should take place at least in the warmer months in the northern, and in the colder months in the southern part of its range. Both sexes apparently spawn at all times of year at Plymouth, and presumably larvae are produced, so a wide range of temperatures should be suitable for development. If low winter temperatures were lethal to larvae further north the spring and summer larvae should still be able to survive. Similarly, further south, the winter-spawned larvae ought to be able to survive. Thus, the northern limit of this species would appear to depend on the effect of low winter temperature on the adults, but there seems little reason for the position of the southern limit. As far as the breeding cycle is concerned *ecaudata* could presumably extend as far south as *branchialis*.

Other species of *Arenicola* do not show very clear correlation between breeding habits and geographical distribution; *A. marina* is a cold-water species which does spawn in the colder months of the year, near its warmer limits (Newell, 1948; Duncan, 1950), but *A. claparedii*, another cold-water species, appears to develop during the cold months and spawn in spring or early summer (Ashworth, 1912; Guberlet, 1934; Okuda, 1938). *A. cristata* is a warm-water species which spawns in summer in the cooler parts of its range, though the gametes develop throughout the winter (Downing, 1911). This suggests a breeding cycle rather like that of *A. branchialis*, with spawning a few months earlier. In the tropics, spawn of *A. cristata* has been reported at various times of the year (Okuda, 1938; Takahashi, 1938; Bhatti & Soofi, 1949), which suggests that, like many tropical animals, *A. cristata* may breed all the year round. Information on the breeding of the remaining species of *Arenicola* is not sufficient for comparison with their distribution.

Before we can come to any definite conclusions we need to know more about the breeding of all the species of *Arenicola*. Temperature can be only a partial cause of the limited distribution of *A. ecaudata*. The limits of *A. branchialis* seem, however, to show the effect of temperature on various stages of the life cycle. The boundary between these two species might be affected by the influence of temperature on competition between them. Although *ecaudata* is commoner than *branchialis* at Plymouth, the latter maintains itself by a short spawning period compared with the almost continuous spawning of *ecaudata*.

The effect of parasites must also be considered; it is possible that increasing temperature might favour the species of *Gonospora* at the expense of their host and that farther south *A. ecaudata* might be rendered completely sterile by heavy infections of these sporozoans.

From anatomical evidence Ashworth (1912) considered *A. ecaudata* to be the species of *Arenicola* nearest the ancestral form, and perhaps the most ancient of the existing species. If this be so, its geographical range might have been wider in the past and have become restricted by competition, parasites or other causes, in comparatively recent times.

SUMMARY

The two 'tail-less' lugworms, *Arenicola ecaudata* and *A. branchialis*, live in gravel and under stones, on the shores of western Europe. *A. ecaudata* has a more boreal range than *A. branchialis*, but both have a restricted geographical range compared with the remaining species of *Arenicola*, which live in sandy beaches.

At Plymouth *A. branchialis* is a winter-spawning species. Development of the gametes continues through spring, summer and autumn, and spawning apparently occurs over 4–6 winter months, usually November to February.

A. ecaudata, on the other hand, apparently spawns all the year round, mature males and females being found in all months. In 1954 and 1955 there was a fairly definite breeding cycle, with development of gametes in spring, followed by several periods of spawning and redevelopment until the end of the following winter. There seems to have been no cessation of spawning at all in the early spring of 1956; periods of redevelopment and spawning were practically continuous from winter 1955–56 to winter 1956–57.

Only *A. ecaudata* is infected with sporozoan parasites, which frequently reduce its fecundity.

Larval development of *A. ecaudata* was followed up to the appearance of the 6th setigerous segment, when the adult pattern of behaviour was already beginning to appear.

The distribution of *A. branchialis* may be controlled by the effect of temperature on various stages of the life cycle. The northern limit of *A. ecaudata* can likewise be explained by temperature limitation, but the restricted southern limit may be due to effects of competition, parasites or other causes; it is apparently not due to a direct effect of temperature on the breeding cycle.

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OBSERVATIONS ON THE GYMNOSOMATOUS PTEROPOD *CLIONE LIMACINA* (PHIPPS)

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(Text-figs. 1–3)

The Pteropoda Gymnosomata are fast-swimming, shell-less, carnivorous opisthobranchs. The single representative taken at Plymouth is usually regarded as the sexually mature dwarf form of *Clione limacina* (which replaces the typical boreal form of the species in warmer North Atlantic waters). It turns up frequently in the summer and was first shown by Lebour (1931) to breed in these waters. The material used in this account was taken alive near Station EI in August, 1953, and was supplemented by a collection very kindly made for me on the cruise of R.V. *Sarsia* in the Celtic Sea, in September, 1956, by Drs J. and Sylvia Gilpin-Brown to whom I am greatly indebted.

Workers on gymnosomes have suffered more than most from the difficulty of seeing well fixed and living material. It was, therefore, thought worthwhile briefly to examine some features of the morphology of this common pteropod that are not adequately known, and to give some notes on its habits in life. After Pelseneer's classic work (1888) came Meisenheimer's 'Valdivia' report (1905), our finest store of information on Gymnosomata. Later workers such as Tesch, Pruvot-Fol, Bonnevie and Massey have concentrated chiefly on the important task of taxonomy, and many details of the morphology and habits are necessarily passed over.

Plymouth specimens of *C. limacina* show well recognizable sexual development at 2·5 mm long. Lebour (1931) found eggs being laid by individuals of 5 mm. Like so many opisthobranchs, *Clione* is protandrous. Fig. 3C (p. 293) shows the size distribution of a sample of 338 specimens from Dingle Bay, west coast of Ireland, September 1956. The smallest size-group (2·5 mm length when fixed) already showed dividing spermatocytes and fully formed sperm in the ovotestis. Only a few undeveloped oocytes were present, as tiny inconspicuous cells at the periphery. Specimens of 3·5 mm length had most of the sperms mature and stored also in the hermaphrodite duct (see Fig. 3B), while in the largest of the September sample, oocytes were numerous and large (*ca.* 0·8 mm) but no eggs appeared ready to be shed. Even the largest specimens kept the posterior ciliated girdle, a characteristic feature of the dwarf race. The colour was yellowish white and the red patch of the gonad was less intense than in the typical 'northern' form. There is a further difference in the radula, the dwarf form having fewer lateral teeth, as was shown by Lebour (1931) (see also M'Intosh, 1898).

C. limacina swims gracefully, either rising vertically or moving forward with the body horizontal. The locomotor organs are a pair of wings set transversely at the anterior fourth of the under side. They are narrowly attached at the sides of the mid-line and expand to form broadly rounded flaps. As in all pteropods they are modified parapodia, or outgrowths of the side of the foot. The median part of the foot is vestigial, remaining near the centre in the form of one central and two lateral lobes (Fig. 1, *m.f.*, *l.f.*). Observations on swimming were made from Plymouth material, and I am particularly indebted to Dr J. Gilpin Brown, who observed living Irish specimens, for helping me with the diagrams in Fig. 1. The wings move synchronously dorsally and ventrally,

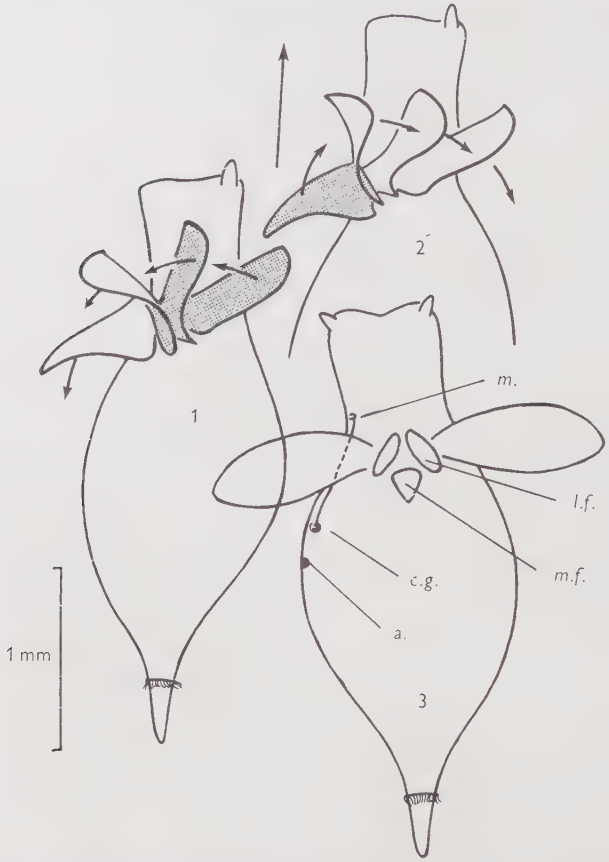

Fig. 1. Outline sketches showing the successive positions of the wing in downward (1) and upward (2) strokes (left-side view), and (3) ventral view. *a*, anus; *c.g.*, common genital aperture; *l.f.*, lateral lobe of the vesigial foot; *m.*, male aperture; *m.f.*, median lobe of the foot.

that is towards and away from the sides of the body. While the smaller thecosomatous pteropod *Limacina retroversa* (see Morton, 1954) uses its long wings as oars for rowing, *Clione* employs its shorter wings for sculling. At its narrow attachment to the body the wing makes a small twist after each stroke so that the leading anterior edge is directed relatively more strongly downwards in a down-stroke and upwards on the return. The effect of this is that the ventral surface of the wing faces posteriorly after a down-stroke and the dorsal surface is inclined backwards after recovery. Each stroke is thus given a backward component; while moving ventrally the wing has an upward as well as a forward thrust, compensated by a downward thrust as well as a forward thrust on moving upwards. The beating of the wings is much more rapid than in the thecosomatous pteropod *Limacina retroversa*, and movements are generally much faster, and the power of quick manoeuvre greater. With the ability to utilize both upward and downward strokes in a sculling action may be related the shorter, rounder wings, and the smaller wing surface found in gymnosomatous pteropods.

The body is perfectly streamlined and *Clione* has no external gills or excrescences of the skin. Respiration appears to take place through the general body surface, and the whole body cavity forms a wide, blood-filled haemocoele. The circulatory system is extremely simple. The heart, as first described by Meisenheimer (1905), is enclosed in a thin pericardial cavity lying at the posterior part of the visceral mass on the right side. The renal organ, which opens close to the anus, is a thin transparent sac; its walls are neither folded nor glandular.

Like most pteropods, *C. limacina* has the eyes reduced; I could find none at all in serial sections of Plymouth specimens. Gymnosomes, however, find no difficulty in catching zooplankton, and have been generally observed and assumed to feed on thecosomatous pteropods. *C. limacina*—as with both Plymouth and Irish specimens collected here—generally accompanies larger numbers of *Limacina retroversa*. The gymnosome buccal mass bears a fine array of hooks, spines, exsertile radular teeth, and adhesive tentacles (cephaloconi) or tentacles bearing suckers (acetabula). These pteropods thus as a group combine some of the efficiency of both cephalopods and chaetognaths. The Clionidae have no acetabula nor jaw, but possess cephaloconi, arranged in a circlet just within the mouth—in *Clione limacina* three at either side. There is also a pair of long hook sacs, one opening at either side of the pharynx, containing an invaginated cluster of chitinous 'hooks', in this species really slightly curved blades, about fifteen in number. Fig. 2B is from a specimen with its buccal armoury everted. So far as is known, the act of food capture has never been described. Observations on this and other habits of the fine gymnosomes of colder seas would be very rewarding.

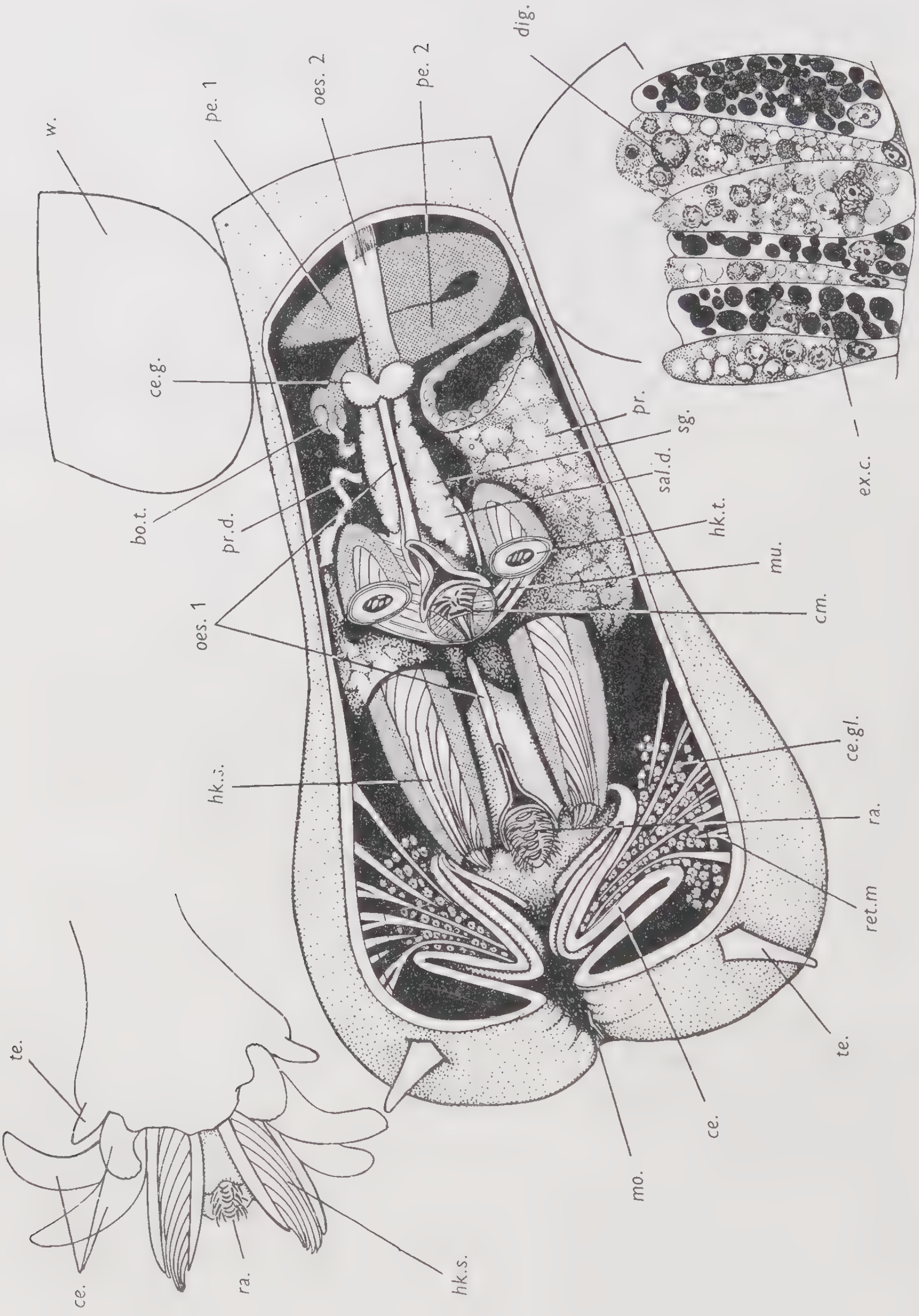
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Fig. 2. For legend see opposite page.

ALIMENTARY CANAL

Fig. 2A explains the structure of the buccal mass. The mouth is a distensible vertical slit, and the prehensile organs—cephaloconi (*ce.*), hook sacs (*hk.s.*) and radula (*ra.*)—lie within it, and are everted, in that order. The three cephaloconi on either side are withdrawn into a recess at the side of the buccal cavity when out of use. The epithelium of the cones carries rod-like sensory cells, and their cavities and the adjoining cephalic haemocoele are filled with clusters of unicellular glands, having long ducts and staining blue in azan. Their secretion is assumed to be adhesive (see also Pelseneer, 1885). Slips of retractor muscle extend into the cones from the body wall, while they are evidently extruded by the pressure of blood. The hook sacs are ovoid to cylindrical tubes, longer than the pharynx in *Clione* and opening into it at the sides of the odontophore. Hooks of gradually increasing size are inserted on the mesial wall of the sac, each with a secretory cell underlying it, so arranged that all, including the shortest, protrude into the pharynx. The wall of the sac has a complex musculature, circular outside and longitudinal round the lumen. The hooks can evidently be everted and spread out rather more freely than can be appreciated from Fig. 2B, where the hook sacs themselves are probably extruded rather beyond the natural distance from the mouth. The radular sac runs back between and slightly below the hook sacs, lying in a groove above the two-rod-like cartilages which form the body of the odontophore. The teeth are well figured both by Pruvot-Fol (1926) and Tesch (1950). Slight differences in the serrations of the median tooth in the figures of various authors are to be attributed to different amounts of wear in the teeth at the front of the radula. The main prehensile teeth are those of the lateral rows, which can be strongly erected as the radula protrudes. The salivary glands (*sg.*) are long straps running forward to open into the pharynx at the sides of the radular sac. These pass with the oesophagus through the nerve ring. Their cells are filled distally with granules staining black in haematoxylin and red in azan. Subepithelial

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Legend to Fig. 2

Fig. 2. A. Stereogram of the head of *Clione limacina*, opened to show the buccal organs and the male genitalia lying within it The buccal cavity has been opened, and the oesophagus, radular sac and hook sacs cut across in transverse section. The distal part of the prostate gland has been removed, to display the penis sheath lying ventrally to it. Except for the cerebral ganglia, details of the nervous system are left out. × *ca.* 90. B. The buccal organs protruded from the mouth. Drawn from a preserved specimen. C. Histology of the digestive gland. *bo.t.*, 'bouton terminale', where prostatic duct opens into penis sheath; *ce.*, cephalocone; *ce.g.*, cerebral ganglion; *ce.gl.*, glands at base of cephalocone; *cm.*, radular caecum seen in section; *dig.*, digestive cell; *ex.c.*, excretory cell; *hk.s.*, hook sac; *hk.t.*, hook sac in section; *mo.*, mouth; *mu.*, transverse muscles between hook sacs; *oes.* 1, anterior part of oesophagus; *oes.* 2, posterior part of oesophagus; *pe.* 1, part of the penis sheath containing the penis; *pe.* 2, prostatic part of the penis sheath; *pr.*, prostate; *pr.d.*, duct of the prostate; *ra.*, radula; *ret.m.*, slips of retractor muscles of the cephalocones; *sal.d.*, salivary duct; *sg.*, salivary gland; *te.*, head tentacle; *w.*, base of swimming wing (cut short).

mucus glands open through the sides and floor of the buccal cavity in front of the odontophore.

The rest of the alimentary canal is extremely simple. The oesophagus is narrow, but with deep extensible folds, and leads back to a large stomach-like sac. This bag is formed by two massive brown or blackish coloured digestive diverticula; the posterior one is larger, and extends farther back, especially on the right upper side, and the smaller diverticulum opens from its deeper aspect as a pocket. The true stomach—as in most carnivorous opisthobranchs—is reduced, but to a greater extent in *Clione* than described anywhere else. It forms a mere funnel of ciliated epithelium on the right side of the digestive sac from which the short intestine runs forward to the body wall. The intestine is a narrow, strongly ciliated tube, and the relatively slight faecal waste is discharged without any mucus binding.

The digestive epithelium with which the 'stomach' is lined has two types of cell (Fig. 2C). The first is long and columnar, with the typical histology of molluscan absorptive-digestive cells. The cytoplasm contains vacuoles filled with particles of varying sizes staining purplish blue in azan, and especially concentrated near the broader free ends of the cells, where there is much diffuse material taken up from the lumen. The intact cell has a narrow striated border. Small spherules from it are constricted off at times into the lumen. The other type of cell, less than half as numerous, is filled with rounded spherules, jet black in haematoxyiin, bright red after azan. On general grounds these cells are probably enzyme secreting; from the nature and bulk of the meal, there is obviously much extracellular digestion, and the gut has no other likely source of secretion. The only alternative function to be considered is excretion, since the kidney, a smooth oval sac lying on the right side, has lost all its glandular character. Amoebocytes, however, are distributed subcutaneously, and it may be that these—as in many other molluscs—are able to eliminate waste directly through the body wall.

REPRODUCTIVE SYSTEM

The genital ducts of *Clione* have never been described in detail. Fig. 3A illustrates the form and arrangement of the hermaphrodite reproductive system, which is laid out on the plan of primitive opisthobranchs. The ovotestis is a conical sac lying behind the other viscera. Its central part is chiefly a testis, and in older animals (3 mm and above) the periphery is crowded with small acini containing developing oocytes. A narrow hermaphrodite duct, which can be distended as a vesicula seminalis for outgoing sperm, leads forward to the glandular ducts, which fall into two parts. The first is the small, flattened albumen gland (*alb.*) into which the hermaphrodite duct opens directly, and through which both ova and sperm must pass. It is lined with large squarish cells, staining pink at the base and with the secretion spherules blue after azan.

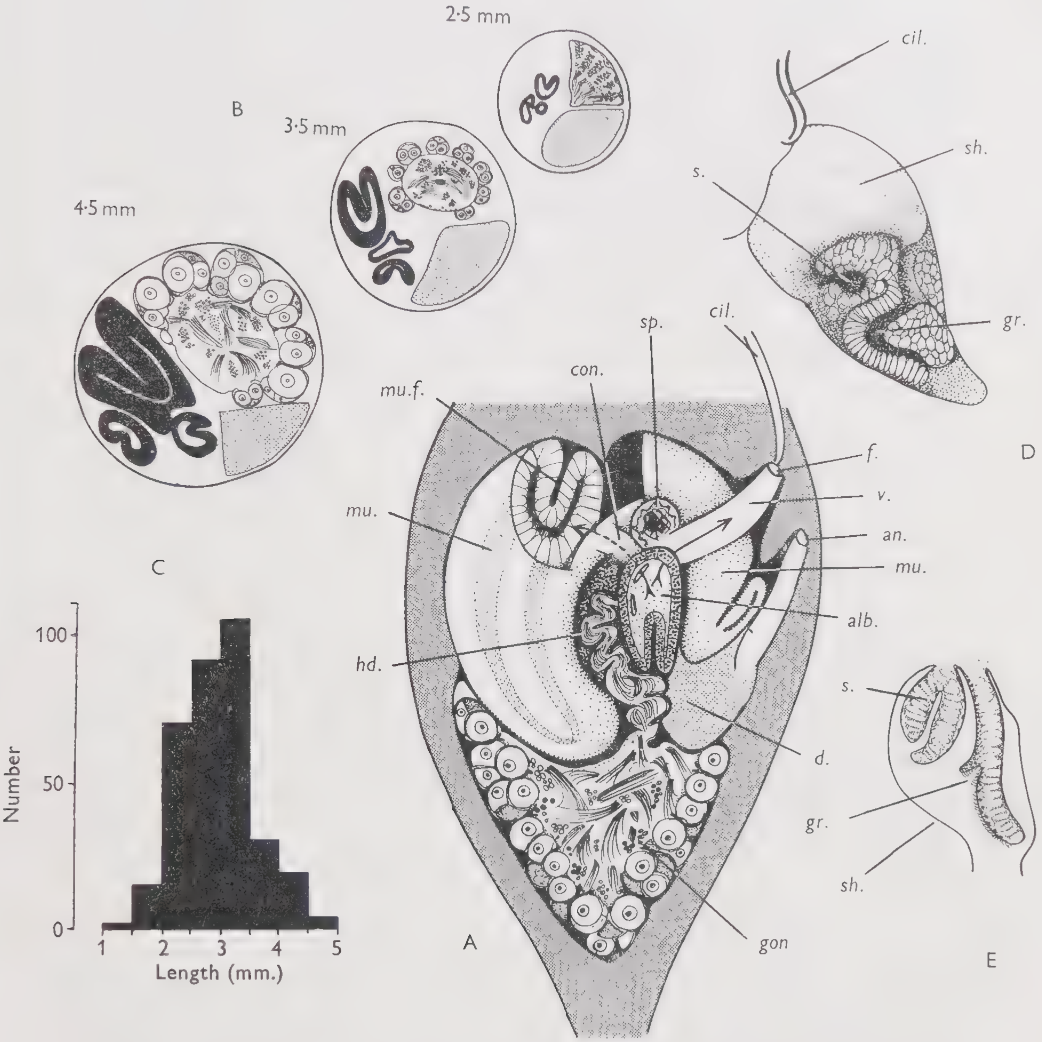

Fig. 3. A. Stereogram of the gonad and genital ducts, in relation to the outline of the body. The gonad and albumen gland are viewed as by transparency, and the mucus gland is cut across to show its internal fold. A more anterior portion of the gonad overlies the genital ducts and has been removed. × 10. B. Diagrammatic transverse sections showing the size and contents of the gonad in specimens of 2·5, 3·5 and 4·5 mm length. The genital duct is shown in black, the digestive gland stippled. C. Histogram showing size distribution of a September sample of 338 individuals from the Celtic Sea. The lengths were measured after preservation in formalin. D. Structure of the penis, everted together with part of its sheath. E. The penis within its sheath, in longitudinal section. *alb.*, albumen gland; *an.*, anus; *cil.*, ciliated seminal groove running to male aperture; *con.*, connecting duct between the albumen and mucus glands; *d.*, portion of the digestive gland, after removing the rest of the alimentary canal; *f.*, common genital aperture; *gon.*, gonad; *gr.*, seminal groove running along the penis; *hd.*, hermaphrodite duct, filled with sperm; *mu.*, mucus gland; *mu.f.*, internal fold of the mucus gland; *s.*, sucker-like appendage of the penis; *sh.*, penis sheath; *sp.*, pouch attached to the vagina, containing sperm; *v.*, vagina.

Scattered ciliated cells lie here and there around the lumen. This gland evidently lays down the thin nutritive coating round the eggs, before they pass through the mucus gland (*mu.*). This is a large horse-shoe shaped caecum, blind at either end, and opening narrowly from the albumen gland. Its cavity is divided by a broad fold. When fully secretory, the cells are massively distended and colourless. At the surface are a few ciliated cells which, together with the weak muscular action, may help move the contents. The dimensions of the mucus gland correspond very well to the oblong gelatinous strip (1–1·2 mm long), in which the eggs are embedded, described by Lebour (1931). One egg strip with some thirty to fifty eggs evidently fills the gland at one time.

Fom the albumen gland a narrow vagina strikes out to the body wall on the right side. Near it lies the anus and renal opening; and through it pass both eggs and outgoing sperm. The latter travel forward by an external ciliated groove above the right wing, to enter the male genital pore which leads to the ensheathed penis. For incoming sperm, the vagina bears no stalked bursa copulatrix; but in a mature female serially sectioned was found a pouch from the vaginal wall, with sperm in its lumen, and disorganized chromatic material in the cell cytoplasm, apparently from sperm absorption (see Fretter, 1942).

Boas (1886) figured two *C. limacina* in copula from spirit material, but the functioning of the male organs has never been made clear in the literature. Boas shows the sexual act as reciprocal and simultaneous, the two pairing venter to venter with the heads facing the same way. The two extruded penes crossed to reach the female apertures of the partners. The extruded penis is illustrated in Fig. 3D. It is a fairly robust, roughly conical structure, not inversible but merely invaginated when out of use into the penis sheath. The figure shows it attached at the base to the penis sheath, more of which has been evaginated than is likely to happen in life. The surface of the penis is deeply furrowed, and the main groove (*gr.*) is ciliated and forms the sperm channel. This grove is probably continuous during copulation with the terminus of the ciliated external groove. The penis is built up of stiff, vacuolated, cartilage-like tissue, and some distance behind its pointed tip, is a cup-like or flattened area which has the appearance of a sucker. This is shown in section in Fig. 3D, and in section in Fig. 3E.

Wagner (1885) gives an odd account of copulation, quoted by Pruvot Fol, in which he claims that an individual *B* takes semen from a partner *A* and hands it on to a third individual *C* (!). Fresh study of copulation in life is much to be desired, but even from careful anatomical study, it would seem that some of the things claimed in the literature are very unlikely. Eschricht (1838), followed by several others, figured the penis with a second branch, a long slender arm carrying a 'sucker' at its tip. This structure—which I have found everted in several preserved specimens—seems to be merely an artefact due to the over-evagination of the penis sac, carrying with it part of the prostatic duct. This duct opens into the penis sheath by a button-like sphincter called by Kwietniewski (1903) the 'bouton terminale', which has been regarded as the 'sucker' when evaginated. It is unlikely that such evagination ever happens in life, and the whole 'arm' is thin and flaccid, certainly useless as a prehensive organ. If indeed the copulating animals must secure a firm hold on the smooth body of the partner, the shallow acetabulum on the body of the penis would be better adapted for this role.

The prostate evidently contributes its secretion to the semen by pouring it into the penis sheath, close to the base of the male organ. It opens there by a narrow, slightly coiled duct, discharging through the sphincter (*bo.t.*) called the 'bouton terminale'. The prostate itself is a massive strap-shaped appendage, extending forward from its duct, then transversely beneath the buccal mass, and finally backward on the left to fill all the space dorsal to the penis sheath. The gland was termed the 'foot-gland' by Pruvot-Fol (1926) but was correctly recognized by Kwietniewski. Its histology resembles very closely that of *Otina otis*, described by Morton (1955), the cells being very large and filled distally with large blue-staining (azan) aggregates of secretion.

AFFINITIES OF GYMNOSOMATA

Pelseneer (1888) first clearly distinguished the thecosomatous and gymnosomatous pteropods as separate stocks. From various evidence he held that the Thecosomata were specialized bullomorphs, a view that has commanded wide support ever since. The Limacinidae, with multi-spired sinistral shell and operculum, may well have arisen as pelagic neotenic bulloids (see Lemche's account (1948) of tectibranch larval shells). Pelseneer then demonstrated that swimming wings might have arisen by the enlargement of the parapodia seen in *Aplysia*—itself an inexpert swimmer—or in *Akera*, and suggested an aplysioid origin for the Gymnosomata. In his book of 1906 he abandons the Pteropoda as a formal grouping and places his six families of Gymnosomata in the Aplysiomorpha.

Later workers, such as Thiele (1931), Odhner (1939), and Hoffmann (1939) have revived the order Pteropoda, to include both the shelled ciliary-feeding Thecosomata and the naked carnivorous Gymnosomata. Thus, by overstressing the one resemblance in the possession of wings, very great differences are minimized and a wholly unnatural group erected in the Opisthobranchia. But the Gymnosomata, different though they are from thecosomes, are not readily acceptable as aplysiomorphs. The Anaspidea of Thiele (or Aplysiomorpha) are a small group with a well marked aspect and a rather narrow radiation, which—thanks to the work of Eales and others—we can now well recognize. They are bottom-dwelling herbivores, swimming temporarily by parapodia, and with the restoration of the Akeratidae (Morton & Holme, 1955) consist of two families. Of the aplysioid characters of the gut, with a well marked crop, double gizzard and caecum, there is not a hint in gymnosomes. Pelseneer compared the spinose jaw of *Aplysia* with the deep hook sacs of gymnosomes, but of the acetabula, cephalocones and gymnosome radula the aplysioids offer no suggestion. The nervous systems and genitalia (see Eales, 1921, and Pruvot Fol, 1926) are likewise only comparable at the most general level.

Pelseneer placed much reliance of the ability on aplysioids to swim; but we now know that, if any accomplishment of the opisthobranchs can be credited to parallel evolution, it is this one—a specialization met with in primitive members of every group having parapodia (*Arthessa* and *Oxynoë* in the Sacoglossa, *Gastropteron* in the Bullomorpha, as well as in pleurobranchoids). *Akera* provides a model of how gymnosome swimming may have arisen, but denotes no close connexion. The gymnosomes are in short a specialized and very distinct group. It may be better to give both Thecosomata and Gymnosomata the status of separate orders, the course most recently followed by Pruvot Fol (1954). If we are to keep them together it must be a marriage of convenience liable to be broken up as soon as more compatible allies can be found.

SUMMARY

Some features of the digestive and reproductive systems, as well as the habits and swimming of the pteropod *Clione limacina* have been studied from specimens of the dwarf 'southern' race, which occurs at Plymouth. The animal swims rapidly by the sculling action of its two short rounded 'wings' or parapodia. Its buccal mass is characterized by specialized prehensile organs, consisting—as well as the radula—of adhesive tentacles (cephaloconi) and a pair of hook sacs. The rest of the gut is simple, consisting chiefly of a 'stomach' formed by two spacious digestive diverticula, that have replaced the true stomach. The lining includes absorbing-digestive cells and excretory cells. *C. limacina* is a protandrous hermaphrodite. The size distribution of a sample of 338 is shown. The youngest specimens are all males; in older groups developing oocytes are found, and in the largest are eggs not yet ready for shedding (September), as well as sperms. The genital tract is of the primitive opisthobranch form, with a sperm-storing hermaphrodite duct, albumen gland and mucus gland. An external seminal groove leads forward to the penial sheath in the head. The structure of the penis and prostate is described, and some previous views on the nature and functioning of these parts are criticized. Finally, the relationships of the gymnosomatous and thecosomatous pteropods are briefly reviewed, and the use of separate orders is recommended in place of the recently revived single group Pteropoda.

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NOTES FROM THE PLYMOUTH AQUARIUM. III.

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(Plates I and II)

CONTENTS

PAGE
The breeding of *Spondyliosoma cantharus* (Gmelin) 299
The sexual display of *Labrus ossifagus* L. 304
Learning in *Zeus faber* L. 306

The present notes (see also Wilson, 1949, 1953) are mainly concerned with the breeding habits of two fishes commonly kept in marine aquaria. These habits may be familiar to keepers of other large marine aquaria, but I have failed to find any account of them in scientific literature other than a very brief and inadequate description by Raffaele (1898, p. 328) of the nesting of *Cantharus vulgaris* (= *Spondyliosoma cantharus*) in the aquarium at Naples. The scientific names of species mentioned follow the *Plymouth Marine Fauna*, third edition (Marine Biological Association, 1957).

THE BREEDING OF *SPONDYLIOSOMA CANTHARUS* (GMELIN)

The Black Sea-bream or Old Wife (*Spondyliosoma cantharus*) has never been common in the Plymouth sea area, but since 1951 occasional specimens have been obtained throughout the year. Earlier records are not numerous, and certainly for 25 years prior to 1951 no specimens were kept in the aquarium. Since that year a varying number, at present seven, have lived in the largest tank (31 × 9 × 4½ ft. deep) in company with more numerous *Pagellus centrodontus* with which they shoal, and with a variety of other fishes and with *Palinurus vulgaris* and *Homarus vulgaris*. Some of them have been in the tank for several years and since 1953 they have bred regularly. The present account is based on observations made over several years.

From early in the year onwards the males, from time to time, make nests until breeding is over, and occasionally afterwards. When nest-making begins the water is often at about its coldest (8–10° C); spawning takes place when the water is about 12–14° C. May is the usual month for spawning, but in 1957 the first spawning took place on 17 April, to be followed by others late in May. The winter had been mild and the water temperature of about 13° C was two to four degrees higher than usual for mid-April. In 1956 spawning was delayed to mid-June, although temperature during the earlier months of that year had not been abnormal and spawning temperatures had been reached in May.

The nest is simply an area of the slate floor of the tank cleared of its usual covering of small pebbles. The male makes the clearing by swimming close above the bottom and exerting vigorous side-to-side movements of the tail, thus creating a backward current that sweeps away the pebbles. He remains upright, head more or less towards the centre of the patch as he clears it and travels thus around the edge of the clearing, not necessarily continuously but usually with pauses. In the early part of the year these nests, which are roughly circular, may be only a foot or so across, but as the breeding season approaches the bared patch reaches a larger size and may have a diameter of 3 or 4 ft. (roughly 1 m). Two or more males may each make a nest at the same time, a few feet apart. They will threaten and chase one another, sometimes facing mouth to mouth, nearly touching, fins erect and bodies flushed almost black with vertical white stripes. Little actual fighting between males has been seen.

Out of the breeding season males and females are practically indistinguishable. In the well-lit tank they are silvery, very pale violet on their upper sides and back, with broken horizontal stripes. It is only in the excitements of nest-making, threatening other males, mating and guarding the eggs that the males justify the common name of 'black'. In almost an instant they can when excited change from the normal pale coloration to an intensely dark violet, almost black, with a very prominent vertical white stripe on each side, just in advance of the anal fin (see Pl. I, fig. 1). There are often similar but paler vertical stripes before and behind the main one; another occurs about the level of the pectoral, and yet another at the base of the caudal fin. There are horizontal dashes of white along the lateral line and small whitish blotches more or less all over, but especially on head and shoulders. Broad horizontal banding may occur on the snout and gill-cover, below eye level. There is much variation between individuals, some never become as dark as others, and the same individual may vary from time to time. All individuals exhibiting this dark coloration and this colour pattern are judged by their actions to be male.

As the combined shoal of *Spondyliosoma* and *Pagellus* swim up and down the long tank, males with nests swim after and dart at others of their own species, assumed to be female, as they pass by. During the early months of the year there is no response from them and the males do not stay long on the nests they have made, soon leaving to shoal up again with the other fish. But every now and then the urge to nest-making drives them to repeat the performance.

When a female is ready to spawn she will accompany a soliciting male down to his nest. Spawning has always taken place very late in the evening or very early in the morning, possibly even at night though there is no evidence for this. The actual spawning has rarely been seen; it has been watched once or twice at about 10 p.m., though not by a scientific observer and details are vague. I have seen, during the daytime, a female accompany a male, which had approached her, to the nest and stay there for some minutes testing the bared slate with her mouth and body. Meanwhile the male swam actively about her, not attacking but now and then nuzzling her ventral fin and her anal region. He was intensely black with prominent white stripes, and he repeatedly erected his dorsal fin. The female bore normal pale coloration. Unfortunately, there was no spawning and as the female swam away she was immediately followed by the male, fussing about her, though as far as could be seen she was not bitten by him.

On another occasion, the following year, a female which had accompanied a male to his nest darkened in colour, though not to the same extent as the male. On the side of her body, behind and above the insertion of the pectoral fin, there appeared a pale horizontal rectangular patch and there were a few irregular thin vertical stripes near the root of the tail. The horizontal pale patch on the female contrasted sharply with the vertical stripes in the same region of the almost black male. The latter became wildly excited and with all fins erect made rapid protrusions of the mouth. Unfortunately, the female stayed on the nest for only a minute or so and no eggs or milt were shed. The performance was repeated several times, for each time the female left she was chased by the male and induced to return, until finally she would go back no more.

The eggs are stuck in a single layer to the slate, although here and there a second layer covers the first. They form an irregular whitish patch a foot or so in diameter in the middle of the cleared area of slate, which is 3 ft. or so across. Each egg is about 1 mm in diameter, flattened underneath and flattened at the sides where it sticks to neighbouring eggs. The egg capsule is relatively tough. The developing embryo shows a single yellow oil globule about 0·25 mm in diameter. Hatching takes place in about 9 days at about 13° C. The larval fish retains the oil globule in its yolk sac and it swims right way up. The eye is darkly pigmented before hatching.

The eggs are guarded by the male until they have hatched. Whilst on guard he endeavours, especially during the first 2 or 3 days, to induce other females to add to them. In May 1954 a second spawning definitely took place on one nest within 24 hr. of the first, and presumably by a second female. There are less definite indications of double spawnings having taken place in other years. In May 1957 two males each had a nest with eggs at the same time, one lot of eggs being a day older than the other.

The first duty of the guarding male appears to be to keep the eggs clean and to prevent them from being silted over. At frequent intervals he swims slowly just above the eggs, wriggling his body and fanning vigorously with tail and fins. This action disperses silt, and also pebbles which have been scattered among them by such accidents as a ray, or flat-fish, settling down close beside the nest and with flapping movements attempting to bury itself in the gravel. The male also goes around the borders of the nest, head towards the centre, and widens it by swishing with his tail, just as he does when making it originally. He also picks up pebbles in his mouth and drops them well to the side of the nest. This action is not pursued very effectively for the pebbles have never all been cleared away even when there were only a few on the eggs, and whilst swimming around with one in the mouth the fish occasionally let it fall back again on the eggs, especially when his attention was distracted by the approach of other fish.

The second duty of the male is to guard the eggs (Pl. I, fig. 1). In a tank with so many other fishes and invertebrates this is always an arduous task. Especially troublesome are the various species of *Raia*, the flat-fishes (*Pleuronectes platessa* and *Microstomus kitt*) and the rock-lobsters (*Palinurus vulgaris*). Large wrasses (*Labrus bergylta*), the shoals of common sea-bream (*Pagellus centrodontus*) and of mackerel (*Scomber scombrus*) give little trouble and neither do the pollack (*Gadus pollachius*), although if any kind of fish swims too low over the nest it is likely to be bitten. The most troublesome fish actually observed was a pout (*G. luscus*), of which more later. The reactions of a guarding male towards others of his own species varies with their sex. He attacks any male coming near and may chase after him for some distance; as already described he solicits any female and while following her deserts the nest for a short time.

With the exception of a small sting-ray (*Dasyatis pastinaca*) which on one occasion appeared to be feasting on the eggs (observation by Mr G. R. Forster) and was removed to another tank, none of the other fishes in the tank, not even the pout, were ever seen to attempt to eat the eggs. The offence of the rays and flat-fishes was to lie on the eggs, or on any part of the cleared area. They appeared to settle down on the nest just as they would on any part of the tank floor and seemed indifferent to the eggs. It was while the owner of the nest was away for a few minutes, chasing something else, that this was liable to happen. On his return the intruder was immediately attacked, often swooped down on from above and savagely bitten, on body, fins or even the eyes; this rarely failed to bring about a hurried departure. Once a small ray was seized beside the snout and towed away, not being released until well clear of the nest. Any fish approaching was rushed at and if it did not turn away, bitten. After a few days most offenders learned to avoid the nest and its immediate neighbourhood.

Encounters with rock-lobsters were always amusing to a human onlooker. When a *Palinurus* wandered over the nest the *Spondyliosoma* became very excited. There were several methods of dealing with it. One was to seize an antenna and pull the crustacean off the nest, small ones being lifted well clear of the ground and towed for several feet, until with a vigorous flap of its tail the *Palinurus* pulled itself free. Sometimes the *Spondyliosoma* approached from behind and bit the upper surface of the abdomen, or the tail-fan, whereupon the rock-lobster hurried forward out of the nest. Sometimes the *Palinurus* was pushed from behind, a method adopted more particularly with large individuals with abdomens bent forwards under them, as with females in berry, although rock-lobsters with extended abdomens were pushed almost as readily. The fish applied its mouth to the abdomen of the crustacean and pushed as hard as it could, making very vigorous swimming movements. As it came up behind the *Palinurus* it would take obvious care to avoid the long backwardly directed antennae, coming into the attack between them; it appeared to the observer that it disliked being tickled by them. As far as could be seen only the lips of the fish made contact with the integument of the rock-lobster. The pushing of a large *Palinurus* off the nest entailed much effort.

Of the many fish attacked by the guarding *Spondyliosoma* only one was ever seen to retaliate. This was a pout (*Gadus luscus*) of approximately equal size and unknown sex. It was the only pout in the tank, where it had lived amicably with all the other fish for many months. This pout persisted in haunting the neighbourhood of the nest though not, apparently, with any intention of eating the eggs which did not seem to interest it. It was repeatedly attacked by the black sea-bream and bore on its body marks of the many bites it suffered. The pout often turned on its assailant; more often it would initiate its own attack and savagely bite the black sea-bream. On several occasions it was seen to rush at the bream while the latter was attacking a plaice or a ray lying on the nest; the bream then broke off its attack and fled before the pout. On one occasion the pout repeatedly prevented the bream from attacking a ray lying on the nest (Pl. I, fig. 2) and it was some minutes before the bream got a chance to bite the ray. On another occasion the two antagonists met unexpectedly face to face; for about a second they looked at one another, almost touching mouth to mouth, then they rushed at each other each trying to bite the other in the side. The fight, which was over in a few seconds, ended with the pout chasing the bream right down the tank.

This curious behaviour of the pout was observed both in 1953 and in 1954; by 1955 it was dead. As soon as nesting was over, and the male *Spondyliosoma* had shoaled up again with the other fish, attacks by both parties ceased and they again lived peaceably with one another. It is not known if the male black sea-bream which figured in this drama in 1953 was the same individual as that which played the part in 1954. In the latter year the nesting male was generally much more darkly pigmented than the male which nested in 1953; the two males therefore may not have been the same fish.

The events described took place during daylight hours and it is not known for certain what happened at night. On two occasions after dark the caretaker, Mr W. H. Gladwell, reported that when he had switched on the lights he saw that the male had reverted to non-breeding pale coloration and was swimming with the shoal up and down the tank. Several plaice were lying over the eggs unmolested. Just before darkness had fallen the male had been very darkly pigmented and on guard.

In general, the pigmentation of a nesting male is darkest during the first few days. Later there is a lightening, but there are temporary intensifications of the darkly coloured areas during moments of special excitement, as when attacking intruders. When the male has rejoined the shoal, after the eggs have hatched, he can only be distinguished from others of his species by his frayed fins, evidence of the heavy work he has accomplished.

More fully illustrated accounts of these observations were published in *The Illustrated London News* for 28 August 1954, and in *Neptune* for April 1956.

THE SEXUAL DISPLAY OF *LABRUS OSSIFAGUS* L.

The Cuckoo Wrasse (*Labrus ossifagus*) is not uncommon on or near rocky grounds near Plymouth. Usually one or more specimens, male or female, are to be seen in the aquarium, some of them surviving for several years. Until recently breeding had never been observed, although males had fought one another.

In the spring of 1955 two males and three females shared the same tank. As was usual, the males were hostile but had tolerated one another for some time without undue disturbance. Early in May one of the males was found dead and was believed to have been killed by the other, which had become sexually active and was engaged in nest-building. The nest was merely a cleared area, a few inches across, of the slate bottom of the tank. The bottom was strewn with shell gravel and in order to clear his patch the male turned over on his side and flapped vigorously with his tail (Pl. II, fig. 1). This action should be contrasted with that of the male *Spondyliosoma* which remains upright during the same operation. In nature the fish may in this way clear patches of rock, or make saucer-shaped depressions in sand or gravel. In the tank two separate patches of slate were cleared and so the one male had two nests a foot or so apart. Nest-making took place on several evenings over a period of 1 to 2 weeks.

The nest, or nests, having been made, the male turned his attention to the females, darting swiftly at each in turn, sometimes not otherwise molesting them, but often biting and chivying them. His excitement was great and his colours unusually vivid, the white patch (see p. 305) on head and shoulders visible, though not of maximum prominence. The dorsal fin was fully erect during the attack. The colours of the females were also more pronounced than usual* and had been so for some days. Usually they did their best to avoid the attentions of the male and it was obvious that they were not in full breeding condition. Nevertheless, they were approaching it, for on several occasions a female eventually followed the male to one of his nests. When this happened he became wildly excited and with all fins fully spread, showing the most vivid blue and orange colourings, he displayed himself to her, open mouthed (Pl. II, fig. 2). His head and shoulders were jerked from side to side, his body sometimes twisted into an S-curve. The most striking feature of the display was the blanching of a large patch of skin on top of the head and over the shoulders, the blanching extending a little up the base of the dorsal fin as far back as the sixth or seventh fin ray. From this large, almost completely white area the pigment appeared to have been drained away to leave only faint tracings of the irregular stripes normally present. It has already been mentioned that the whitish patch appeared, with varying degrees of prominence, during the attack on the females; it was also present, though not fully blanched, during nest-building (Pl. II, fig. 1). It completely transforms the normal appearance of the male and must to a ripe female be a visual excitation stimulating her to shed her eggs. Our females, not being ripe, responded only by staying for a few minutes beside the male on the nest, never more than one at a time, showing no excitement and with their colours only slightly heightened. Neither eggs nor sperm were shed, the females soon tiring and swimming away, promptly to be followed and attacked by the male. Occasionally this renewed attack would induce a female to return to the nest, but only for a few moments. Eventually the largest female had to be removed to another tank to save her from the persistent biting of the male. Two smaller females remained in the tank with him; they too were bitten but were not as severely treated as was the larger female. After nearly 3 weeks the latter was returned to the tank, (on 1 June) but almost at once was viciously attacked by the male and had to be rescued for the second time. This last attack took place in the morning, with no nest and with no appearance of the whitish head and shoulder patch, and therefore may not have been an attempt to mate.

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* In the aquarium the colours of healthy but not breeding fish of both sexes are much paler than in living freshly caught specimens, or than in those recently dead.

Sexual displays always took place during the evening, after about 6 p.m. They occurred almost every evening during the first week of May, but were observed only every other evening in mid-May. They then became less frequent. The water temperature during May rose slowly from about 11° C at the beginning of the month to about 13° C at the end. The whitish head and shoulder patch was never visible until the evening; it appeared only when the male was sexually excited and then very quickly. During normal daytime activities the area of skin which it occupied could hardly be distinguished from that surrounding it.

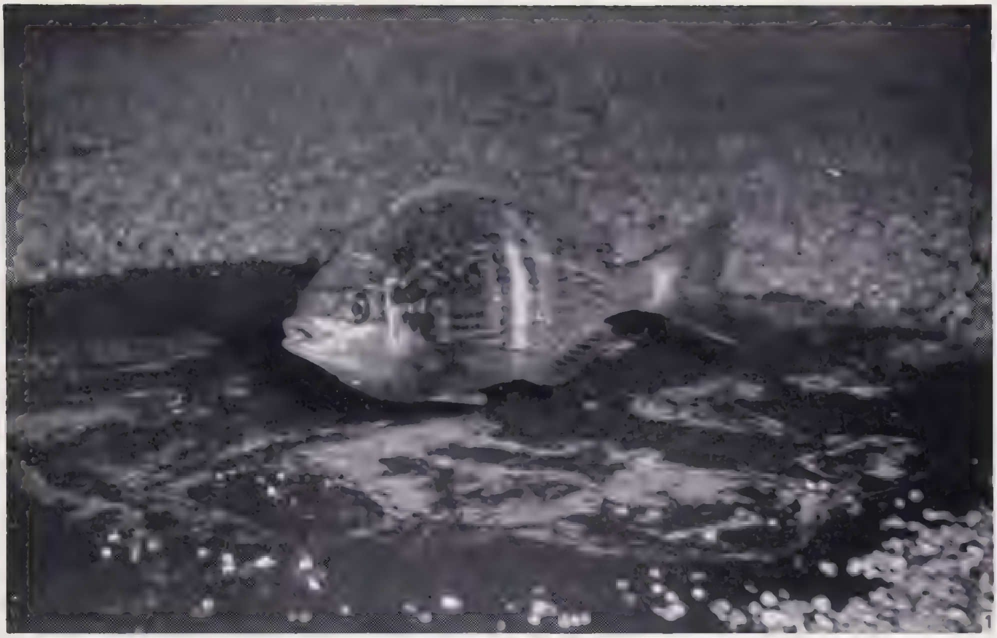
On 9 June the male was suddenly attacked by numerous praniza larvae of *Gnathia* (both *G. maxillaris* and *G. oxyuraea* are known to occur in the tanks). The larvae attached themselves to fins and body and even inside the mouth. The fish became poorly and there was no more sexual activity. The praniza larvae also attacked the females, though to a lesser extent. The fish were removed, freed from the pests, and their tank emptied and cleaned before they were put back in again. The male survived for about another year and the females for two. The trouble with praniza larvae reoccurred months later and in 1957 may have been largely responsible for the deaths of the females.

An account of these observations, illustrated by colour photographs, was published in *The Illustrated London News* for 26 May 1956.

LEARNING IN *ZEUS FABER* L.

The John Dory (*Zeus faber*) is predominantly a fish-eater, stalking its prey until sufficiently close to seize it with a sudden protrusion of the long extensile jaws. For very many years living small fishes, especially *Gobius minutus*, and occasionally living shrimps (*Crangon vulgaris*) when fish were scarce, were fed to the John Dories in the aquarium, and it appeared that they would not accept anything else. Dead fish were almost always ignored and, if perchance taken into the mouth, spat out again. Fish were always carefully scrutinized and a goby passively sinking was almost never seized until it made some movement, though some individual John Dories were less particular in this respect than were others. A few years ago one John Dory starved itself to death, for it would so rarely eat anything, though paying great attention to living gobies put into the tank and staring at one for minutes at a time, while its companions were greedily eating others. Pieces of fresh squid (*Loligo forbesi*), the staple food of the inhabitants of the aquarium, were never taken by the John Dories. Pieces of squid are pure white and are unlikely to be mistaken for fish. A dead goby looks unlike a living one, especially when sinking with silvery ventral side uppermost.

For many weeks after their arrival John Dories have always behaved in this way. Of recent years, however, they have been trained to eat squid as well as dead fish. The training takes a long time and says much for the patience of Mr W. H. Gladwell who is responsible for feeding the animals in the aquarium. After some weeks, when the newly arrived John Dories are feeding regularly on living fish, a few whole small squid or pieces of mantle cut into strips roughly fish-shaped, are dropped into the tank. As the strips sink they do so with an irregular motion, imparted by their shape, and to the John Dories may seem alive. They are scrutinized and eventually, sometimes after many such offerings spread over days or weeks, an odd strip or two will be seized. The diet of living fish can then be gradually stopped until finally only squid or dead fish is given. Mr Gladwell maintains that in giving dead fish the heads should first be cut off. At long last the John Dories feed regularly on squid and no longer need to be offered elongate pieces, square or any other shape will be accepted. At the time of writing (October 1957) six medium-sized John Dories, caught during the summer of 1956, are fed two or three times a week on portions of squid, which are taken eagerly.



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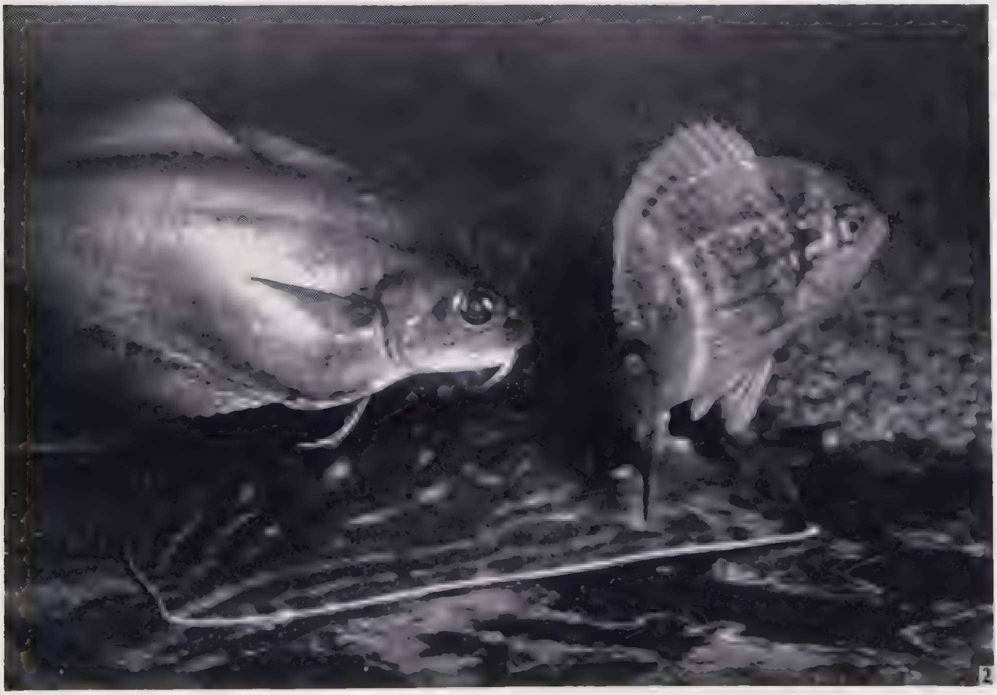

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EXPLANATION OF PLATES

Plate I

Fig. 1. A male *Spondyliosoma cantharus* on guard over his nest (in May 1954) shows breeding coloration developed to about half full intensity. The nest is a cleared area of slate 3 or 4 ft. across; only a portion is shown in the photograph. Unfortunately, the slate bears the marks of old concrete (where rocks were formerly cemented to the tank floor) and the eggs, which when this picture was taken were hatching and losing their whiteness, are not readily distinguishable from this concrete.

Fig. 2. The male *Spondyliosoma* which was about to attack a *Raia microcellata* lying over the eggs, is being headed off by the *Gadus luscus* and it was sometime before the former shook off his pursuer and returned to his task of removing the ray.

Plate II

Fig. 1. Male *Labrus ossifagus* making a nest by lying over on his left side and vigorously flapping his tail to wash away shell gravel, disturbed particles of which are seen behind him. The white area on the top of his head and shoulders is present but not fully blanched.

Fig. 2. Male *Labrus ossifagus* in full sexual display before a female; she is too far to one side to be included in the picture. All his fins are erect, his mouth is open, and the white area on top of his head and shoulders is fully developed. Near the lower right-hand corner about half of the nest is visible. The object in the left-hand corner is an old bottle of dark-coloured glass, with a *Blennius ocellaris* inside. This photograph, reproduced in colour from the original kodachrome, appears in *Marine Life of Coastal Waters* (*Western Europe*) by E. le Danois, translated and adapted by N. A. Holme (London: Harrap), p. 155.

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THE FECUNDITY OF CLYDE PLAICE

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(Text-fig. 1)

In early 1956 and again in 1957 small samples of mature female Plaice *Pleuronectes platessa* L. were collected for fecundity estimates to be compared with Simpson's (1951) results for the North Sea populations, and the data from elsewhere that Simpson summarizes.

The fish were collected on 22 and 27 February 1956 and 7 January 1957 by trawling off Mountstuart House, Isle of Bute, at *ca.* 40 m. The treatment, of the fish was similar to that of the Long Rough Dabs collected for fecundity estimation and which has been described in detail (Bagenal, 1957*a*). This paper on Long Rough Dabs should be consulted for details of the collection and treatment of the fish, and of the laboratory methods for the estimation of the egg numbers and also of the statistical analysis of the data.

I should like to thank the master and crew of M.F.V. *Calanus*, and Miss Sheila Morris who counted the eggs.

RESULTS

The data are shown in full in the Appendix, and summarized in Table 1. The relation of fecundity and length of the plaice is shown in the scatter-diagram (Fig. 1), in which the curves for Simpson's North Sea Southern Bight and Flamborough grounds, and the curve for Kändler & Pirwitz's (1957) Baltic plaice from the Bornholm area, are also shown. Kändler & Pirwitz unfortunately, do not give the raw data for each fish and the curve in Fig. 1 has been calculated from the mean values they give.

From Table 1 it can be seen that the fecundity adjusted to a common length was greater in 1957, but the 'condition' of the fish (as expressed as the expected weight of a 37 cm. plaice) was greater in 1956 (cf. Bagenal 1957*b*). However, neither the general level of fecundity nor the condition was significantly different. This is shown in Table 2 which summarizes the covariance analyses of the regressions of the logarithms of length and weight on log fecundity and also that of log length on log weight, for both sets of data. None of the regressions are significantly different and the data may be pooled. Comparisons with the results of other workers are shown in Table 3. It is clear that the Clyde plaice fecundity adjusted for length is considerably

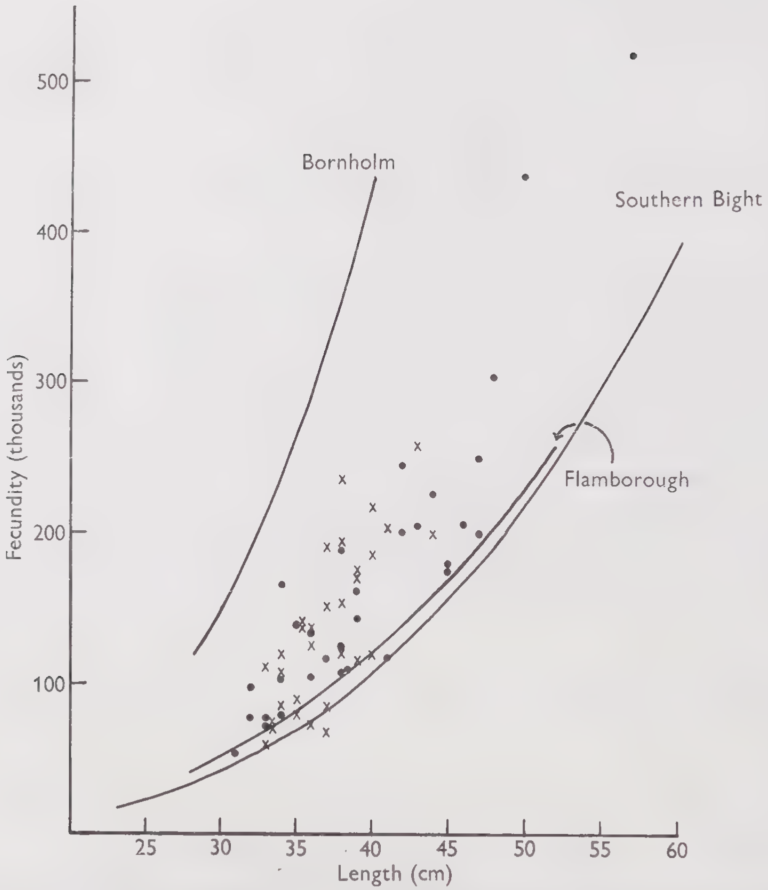

Fig. 1. Scatter diagram showing the relation of fecundity and length of plaice from the Clyde, and the calculated curves for Flamborough, Southern Bight and Bornholm fish (from Simpson, and Kändler & Pirwitz). ●, 1956; ×, 1957.

TABLE 1. SUMMARY OF CLYDE PLAICE FECUNDITY DATA GIVEN IN THE APPENDIX

Year	1956	1957
Number of fish	31	31
Mean length (cm)	39·76	36·91
Mean weight (g)	653·1	518·6
Mean age (years)	5·4	5·1
Mean fecundity	171,080	136,611
$\hat{W}$ for 37 cm	530·7	522·2
$\hat{F}$ for 37 cm	136,693	137,840

higher than that of the North Sea, and much lower than the Baltic plaice fecundity. These differences are statistically significant. Simpson (1957) has recently given a preliminary account of the fecundity of plaice from the Irish Sea; this appears to be similar to that of the Clyde fish given here.

TABLE 2. SUMMARY OF THE ANALYSIS OF COVARIANCE TESTING THE FECUNDITY-LENGTH, FECUNDITY-WEIGHT AND LENGTH-WEIGHT RELATIONS FOR CLYDE PLAICE IN 1956 AND 1957

Source	Fecundity on length	Regression of fecundity on weight	Length on weight
Due to total regression	**	**	**
Difference between means regression and average within subgroups regression	–	–	N.S.
Between adjusted subgroup means	–	–	N.S.
Between regression coefficients	N.S.	N.S.	–

**, indicates significance at 1% probability level.

N.S., indicates not significant at 5% probability level.

–, indicates mean square less than that against which it is tested.

TABLE 3. MEAN FECUNDITY ADJUSTED FOR FISH OF 37 CM, AND THE REGRESSION COEFFICIENTS OF THE REGRESSIONS OF LOG FECUNDITY ON LOG LENGTH

(From data given in this paper, by Simpson, and by Kändler & Pirwitz (1957).)

Author	Region	Date	$\hat{F}$ for 37 cm	Regression coefficient
This paper	Clyde	1956	136,693	3·11
	Clyde	1957	137,840	3·81
Simpson	North Sea Southern Bight	1947/48	82,996	3·13
	North Sea Southern Bight	1948/49	87,152	3·28
	Flamborough	1948/49	96,492	2·85
Kändler & Pirwitz	Kieler Bucht	1952/53	370,954	3·12
	Bornholm area	1952/53	322,771	3·58

Simpson (1951) showed that, among the 1947/48 and 1948/49 Southern Bight fish of the same length, there was only a negligible increase in fecundity with age, whereas for fish of the same age there was a considerable fecundity increase with length. He concludes: 'Thus age alone, apart from its relation to size, appears to have played an insignificant part in determining the fecundity of these fish.' This applied to fish of the same population. On the other hand, when comparing different populations (from the Southern Bight and Flamborough) he showed that the faster-growing Flamborough fish were more fecund. 'These observations lend weight to the view already expressed that fast growing, well fed fish have a higher mean fecundity than slow growing fish.' Age of course comes into this, since fast-growing fish are younger for a given length than slow-growing ones. In Table 4 the mean lengths and fecundities are given for each age group of the Southern Bight, Flamborough and Clyde plaice. Here Simpson's remarks are confirmed and shown to apply to the Clyde fish also, which are faster growing and more fecund than even the Flamborough fish.

TABLE 4. THE MEAN LENGTH AND MEAN FECUNDITY (TO THE NEAREST THOUSAND) FOR DIFFERENT AGE GROUPS OF SOUTHERN BIGHT, FLAMBOROUGH AND CLYDE PLAICE

Region ...	Southern Bight			Flamborough			Clyde		
Age group	No.	Mean length (cm)	Mean fecundity (thousands)	No.	Mean length (cm)	Mean fecundity (thousands)	No.	Mean length (cm)	Mean fecundity (thousands)
II	4	24·4	23	1	31·1	47	—	—	—
III	19	28·4	32	3	32·1	66	3	34·4	84
IV	43	30·5	43	6	34·1	72	16	34·6	108
V	15	32·2	52	3	37·2	78	21	36·5	136
VI	11	35·5	61	2	37·0	87	6	39·9	176
VII	24	37·6	78	1	42·2	96	5	41·6	182
VIII	31	40·2	106	5	42·9	162	2	43·6	209
IX	23	42·9	127	4	45·3	189	1	49·5	434
X	13	43·7	123	3	50·6	194	1	40·0	118
XI	16	42·6	112	3	45·0	129	—	—	—
XII	7	42·6	127	1	51·8	284	—	—	—
XIII	6	46·4	163	—	—	—	—	—	—
XIV	3	47·0	174	1	45·0	144	—	—	—
XV	4	47·7	172	—	—	—	—	—	—
XVI	2	50·3	142	—	—	—	—	—	—

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APPENDIX

TABLE 5. THE LENGTH, WEIGHT, AGE AND EGG COUNTS OF FEMALE CLYDE PLAICE

Fish no.	Total length (cm)	Weight (g)	Age group	Egg count				Fecundity estimate
				1	2	3	4	
22 and 27 February 1956								
1	33·8	443	IV	396	400	382	402	79,000
2	34·4	412	IV	844	801	839	809	164,650
3	40·5	596	Illegible	523	573	639	606	117,050
4	38·2	603	IV	933	948	1028	849	187,900
5	33·4	389	IV	414	327	421	374	76,800
6	42·0	796	Illegible	1039	877	1067	1021	200,200
7	35·8	498	IV	532	481	517	552	104,100
8	39·2	607	VI	814	684	692	672	143,100
9	42·5	845	V	1063	959	1060	1004	204,300
10	46·3	853	VII	1010	1053	1019	1011	204,650
11	48·3	1056	Illegible	1601	1479	1490	1479	302,450
12	49·5	1328	IX	2236	2017	2230	2204	434,350
13	56·6	1743	Illegible	2460	2388	2581	2826	512,750
14	32·1	344	IV	544	443	447	503	96,850
15	38·1	503	V	492	464	540	644	107,000
16	35·4	465	IV	791	609	695	689	139,200
17	31·7	321	IV	486	363	316	378	77,150
18	45·1	792	Illegible	828	921	917	918	179,200
19	37·9	529	IV	671	652	527	622	123,600
20	38·1	535	V	634	696	543	594	123,350
21	46·5	930	Illegible	988	1083	1056	858	199,250
22	39·3	575	VI	871	793	841	707	160,600
23	44·2	812	VII	1060	1105	1189	1150	225,200
24	44·9	772	VIII	975	847	819	843	174,200
25	37·1	491	IV	689	531	595	499	115,700
26	30·5	243	V	283	260	328	206	53,850
27	33·8	365	IV	527	508	486	511	101,600
28	46·7	870	Illegible	1249	1144	1137	1423	247,650
29	42·2	786	VIII	1192	1366	1179	1147	244,200
30	32·8	314	X	378	292	370	375	70,750
31	35·6	431	V	670	622	731	634	132,850
7 January 1957								
1	38·9	545	V	866	841	894	858	172,950
2	34·8	445	V	633	739	682	678	136,600
3	39·9	743	V	1205	1041	1041	1039	216,300
4	34·7	418	V	368	404	378	427	78,850
5	36·5	416	V	512	401	386	379	83,900
6	36·5	518	V	705	744	733	837	150,950
7	37·0	490	III	300	354	277	402	66,650
8	36·2	470	VII	336	369	355	381	72,050
9	33·3	367	III	364	347	402	395	75,400
10	34·2	395	V	550	587	517	482	106,800
11	36·4	480	V	689	628	664	514	124,750
12	38·6	598	V	872	850	789	932	172,150
13	36·4	478	V	704	763	652	644	138,150
14	37·1	594	V	917	968	943	971	189,950
15	33·0	339	III	565	564	514	571	110,700
16	37·8	585	VI	1094	1184	1232	1189	234,950
17	32·7	337	IV	338	340	249	246	58,650
18	32·9	339	IV	360	404	320	333	70,850
19	35·0	447	IV	664	732	658	737	139,550
20	40·0	615	X	633	587	543	587	117,500
21	38·4	544	VI	526	547	618	684	118,750
22	38·7	567	IV	702	583	504	516	115,250
23	38·4	625	VII	681	825	793	755	152,700
24	39·8	684	V	984	974	939	809	185,300
25	34·5	470	V	414	430	429	512	89,250
26	33·8	404	IV	427	427	391	458	85,150
27	33·6	394	V	579	561	562	670	118,600
28	43·0	774	VII	1246	1214	1301	1386	257,350
29	37·7	557	V	871	1146	933	934	194,200
30	43·8	786	VI	1025	943	1090	900	197,900
31	40·7	652	VI	959	1097	1058	943	202,850

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TUBE FORMATION BY *POMATOCEROS TRIQUETER* (POLYCHAETA)

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(Text-figs. 1–18)

The serpulid polychaete *Pomatoceros triqueter* Linnaeus, 1758, with its white calcareous tube, is common on all rocky coasts around the British Isles. Normally 2–3 cm in length, and exceptionally 6 cm, the tubes are found attached to rocks, stones, and shells. Occasionally specimens obtained by dredging in offshore waters have tubes which, although still attached, grow away from the substratum.

This paper is the third in a series on serpulid tube formation. Earlier accounts (Hedley, 1956*a*, *b*) describe the calcium-secreting glands of four British serpulids, together with an account of the organic component of the tube of *P. triqueter*.

DESCRIPTION OF THE TUBE

After a free-living larval stage *P. triqueter* settles on the substratum and secretes a semi-transparent tube (Segrove, 1941). Initially the tube is shorter than the worm and only after a rapid increase in tube length is the animal completely enclosed. Tubes at this stage have thin walls which according to Dons (1927) are non-calcified and which are found by Segrove (1941) to consist of mucus and calcareous matter. Subsequent growth by the addition of calcareous material to the anterior end of this fragile tube results in the heavily calcified tube of the adult worm.

The adult tube is invariably curved in one or more directions and although straight tubes do occur they are extremely rare. On the upper surface there is a keel (Fig. 1), which varies slightly in size and position and which may project as a point beyond the anterior opening. Two other parts of the tube are referred to here as the lateral surface deposit and the anterior surface deposit (Fig. 3). The former is found on each side of the tube mass and the latter is a thin deposit on the substratum at the anterior end of the tube.

A series of ridges on the outer surface of the tube (Figs. 1, 2) give a false and superficial appearance of a series of growth rings. Previously the tube has been thought of as a series of calcareous rings (Potts, *teste* Robertson & Pantin, 1938), a view probably influenced by the surface ridge appearance, and also by the fact that worms removed from their tubes produce hoop-shaped calcareous rings in the fold of the collar.

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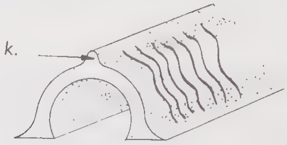
During early spring pure white additions to the anterior ends of the tube develop. These seldom appear in the winter, although if the worms are brought from the shore into the laboratory deposition quickly commences. As a result of such discontinuous deposition there are regions, at irregular intervals along the tube, which indicate cessation (and the beginning) of deposition phases. These are noticeable external features which occur perhaps two or three times along a 3 cm tube. The end of a deposition phase which begins at a level X–Y (Fig. 4) might be expected at a level A–B. This will occur only when both sides of the tube receive an equal amount of calcareous material. In many tubes one side develops to a greater extent than the other, for example, to a level C–D. At the onset of the next phase of deposition the tube will develop as shown only if an equal amount of calcareous material is deposited on both sides.



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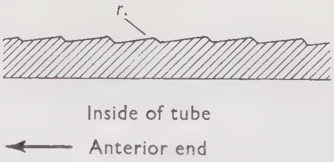

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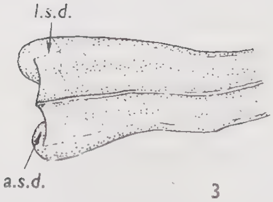

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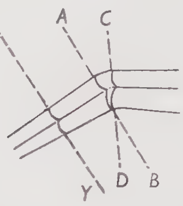

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Fig. 1. Diagram of the anterior end of a tube showing the position of the keel and of the ridges along the tube. *k.*, keel.

Fig. 2. Diagram of a section through the wall of the tube illustrating the ridges seen on the outer surface of the tube. *r.*, ridge.

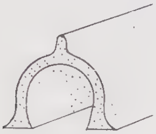
Fig. 3. A top view of the tube. *l.s.d.*, lateral surface deposit; *a.s.d.*, anterior surface deposit.

Fig. 4. Diagram to illustrate a process of tube growth which is discussed in the text.

Tubes are usually incomplete in cross-section and appear as arches over the substratum (Fig. 5). Faouzi (1931) infers from the arrangement of the collar that the worm can never be completely surrounded by the tube. Apparently this is not the case as tubes are sometimes found which are complete in cross-section (Fig. 6). More convincing examples are provided by clusters of

P. triqueter, sometimes dredged in offshore waters, the tubes of which grow away from the substratum and are completely round in cross-section (Fig. 7).

Cavities in the thick lateral portions at the base of the tube (Fig. 9) have been observed and the resulting economy of tube material commented upon (McIntosh, 1923; Thomas, 1940). It has been suggested that other holes which penetrate the side of the tube may be used for the intake and outlet of water for respiratory purposes (Thomas, 1940). These holes, however, are not a regular feature of the tube, and when present, they are few in number and are confined to the early formed and thinner regions of the tube. They are in fact minor variations of the cavities just described, which occasionally are without an external covering and appear as holes in the side of the tube. Only on rare occasions is a hole of this kind continuous through the tube wall into the lumen.



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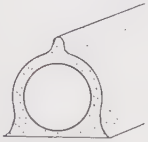

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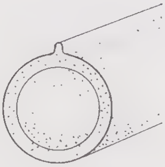

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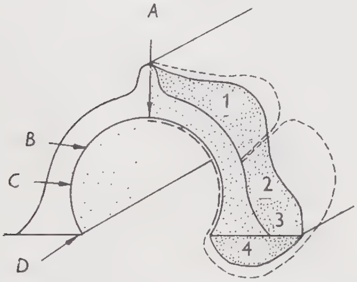


Fig. 5. Diagram of an attached portion of tube which is incomplete in cross-section.

Fig. 6. Diagram of an attached portion of tube which is complete in cross-section.

Fig. 7. Diagram of a portion of tube which has grown away from the substratum and which is completely round in cross-section.

Fig. 8. Diagram of the anterior end of the tube with the broken line indicating the region covered by the collar of the worm (drawn on one side of the tube only). Other areas which are labelled are explained in the text.

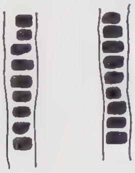
THE MODE OF DEPOSITION

During deposition the worm's branchial crown and operculum project out of the anterior tube opening, and the ventral and lateral collars are folded back over the surface of the tube. In the fold formed by the collar two glands, one on each side of the peristomium, produce calcium carbonate granules, and these together with the secretion of mucopolysaccharide from the unicellular glands in the ventro-lateral peristomial epithelium constitute the material of which the tube is constructed (Hedley, 1956*a*). During the process of deposition the collar mixes the calcium carbonate and mucopolysaccharide, and concomitantly moulds the mixture over the anterior part of the tube.

Observations during deposition can be facilitated by using a tube painted with a quick-drying black paint. Fresh white deposits on these preparations indicate that the regions *A–B* and *C–D* are the first to receive a new deposit (Fig. 8). Following this, further deposits appear on the areas 1, 2, 3, 4, and on region *B–C*. (Fig. 8 is diagrammatic, and where reference is made to an area on one side of the tube it should be understood that a corresponding deposit is also being laid down on the other side of the tube.) Region *A–D* is the free anterior face of the tube; region 1 is covered by the ventral collar; regions 2, 3 and 4 are covered by the lateral collar, and where the lateral collar overlaps on the substratum the anterior surface and lateral surface deposits are found. In addition to deposits on these areas a thin deposit is also laid down on the inside surface of the anterior end of the tube.

The process whereby the length of the tube is increased can be described with reference to Fig. 10. Although all the free anterior surface is receiving new material the upper part receives more than the lateral parts, with the result that the upper part grows forward to a level *X–Y*. Meanwhile the lateral and anterior surface deposits become thicker, but do not advance beyond their limit *L–M* until the worm readjusts the position of the lateral collars. This readjustment is a definite move forward so that with subsequent deposition a very thin anterior surface deposit is found with a limit *O–P*. During this phase the upper part of the tube becomes thicker but not longer. This cycle is now repeated. The whole process involves a relatively rapid forward movement of the collar followed by a stationary phase during which the tube thickens and a small ridge appears on the tube surface. These are the ridges which have been described (Fig. 1).

The presence of cavities in the base of attached tubes (Fig. 9) is probably correlated with the precise arrangement of the lateral collars and the notopodial chaetae of the peristomium. The first sign of a cavity is in position *A* (Fig. 10), where, if direct observation were possible, one would expect to find the notopodial chaetae. These bristles normally move in and out of the chaetal sac and it is suggested that this constant movement prevents the formation of a deposit. With the thickening of the lateral and anterior surface deposits the depression or hole *A* becomes deeper and surrounded by tube material. When the lateral collar moves forward the chaetae do likewise and are no longer associated with the hole just formed. The chaetae now prevent a deposit forming in position *B*, while the hole formed at *A* is covered over by a deposit being laid down on the inside of the tube. This explanation of the formation of the cavities remains speculative in the absence of direct observations.



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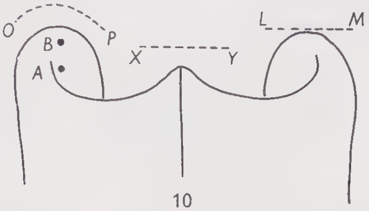

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Fig. 9. Diagram of part of a tube with the roof removed to show the cavities which are present in the base of each side.

Fig. 10. Diagram of a top view of the anterior end of a tube; for an explanation of the lettering see text.

THE POSTERIOR CALCAREOUS GRATING

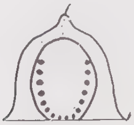
The tubes of *P. triqueter*, in the intertidal zone, are very often damaged at the posterior end. Almost invariably, in such cases, a single calcareous grating or plate (Fig. 11) is present inside the tube, just anterior to the damaged region (McIntosh, 1923; Thomas, 1940).

After damaging the posterior end of a tube, and removing the debris, a grating is formed after approximately 6–24 hr. The first stage is the secretion by the worm of a mucous septum inside the posterior end of the tube. This is followed by the appearance of a number of thin calcareous ribs in the mucous septum (Fig. 12) which become thicker and opaque, while the mucus between them becomes translucent and white (Fig. 13). Calcification continues (Fig. 14), and finally the typical grating is formed (Fig. 11) almost closing the posterior end of the tube.

The grating is a curved structure and this is correlated with the ventral flexure of the posterior end of the abdomen as the worm lies on its back in the tube (Fig. 15). The flexed region of the abdomen acts as a mould in which the calcareous grating is formed. The transverse calcareous ribs correspond in position to the intersegmental grooves (Fig. 16), while the main dorso-ventral rib corresponds to the mid-ventral ciliated groove of the abdomen. The holes in the grating correspond in position to the lateral swollen neuropodia of the abdominal segments (Figs. 16, 18) from which relatively long chaetae project and prevent the complete calcification of the grating.

Attempts have been made to locate the cells or tissues responsible for the secretion of the calcareous material required for the production of a grating.

Worms were fixed in a neutral solution, either ethyl alcohol or an alcohol-formalin mixture, and longitudinal (Fig. 17) and transverse sections (Fig. 18) of the posterior end of the abdomen were treated with the following methods. Staining with toluidine blue for the detection of mucous cells (Sylven, 1941); staining with purpurin (Lison, 1936) and gallamine blue (Stock, 1949) for the detection of calcium; and incineration to locate calcium ash. Negative results are obtained after staining with the lake-forming dyes purpurin and gallamine blue. Other sections stained with toluidine blue and compared with alternate sections, which are incinerated, show that the ventral epithelial mucous cells contain a large amount of calcium. These cells are very numerous in the ventral epithelium compared with the dorsal epithelium where they occur only occasionally. Further comparisons of stained and incinerated sections were made in an attempt to find cells which do not contain mucigen and which have a high calcium content. Such cells were not demonstrated.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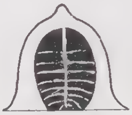
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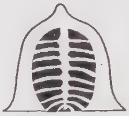
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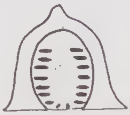
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Fig. 11. Diagram of a calcareous grating which is found inside, and almost closes, the posterior end of the tube.

Figs. 12–14. Diagrams of three stages in the formation of a calcareous grating.

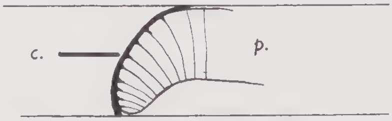
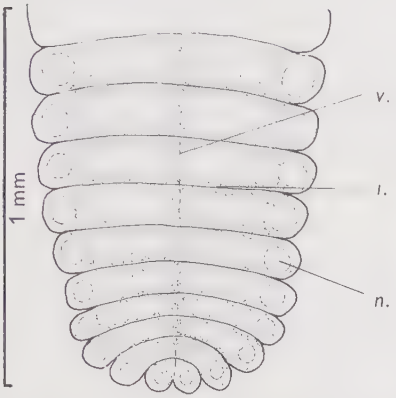


Fig. 15. Diagram to show the ventral flexure of the posterior end of the worm's abdomen and the position of the calcareous grating. *p.*, posterior end of worm; *c.*, calcareous grating.

It is apparent from the position and form of the calcareous grating that the calcium originates from some tissue in the posterior end of the abdomen. It is most unlikely that the dorsal epithelium is involved (bearing in mind that a serpulid lies in its tube ventral surface uppermost), thus the ventral epithelium is most probably the source of the calcareous material. From the results obtained it appears that there are no obvious calcium-secreting organs in the posterior abdomen and it seems likely that the unicellular mucous glands of the ventral epithelium are responsible. It is worthy of mention that the rectum contains a large number of mucous cells and that the calcium-ash content of these is as great as that of the ventral epithelial mucous cells. The contents of the rectum pass out via the anus and then along the ventral ciliated groove, and so the rectum may contribute some material towards the formation of the calcareous grating.



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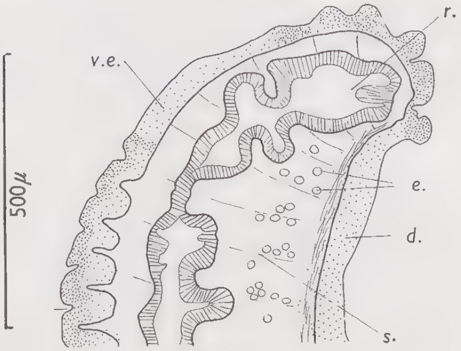

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Fig. 16. Diagram of the ventral surface of the ten most posterior segments of the abdomen. *v.*, mid-ventral ciliated groove; *i.*, intersegmental groove; *n.*, neuropodial swelling.

Fig. 17. Diagram of an oblique longitudinal section through the posterior end of the abdomen. The mucous secreting areas of the epithelium are more heavily dotted. *v.e.*, ventral epithelium; *r.*, rectum; *e.*, eggs, *d.*, dorsal epithelium; *s.*, intersegmental septum.

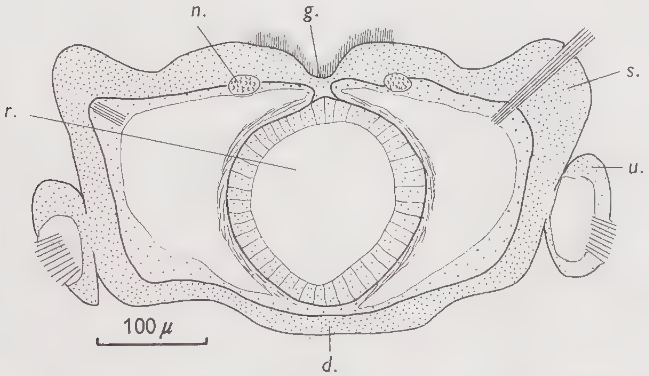

Fig. 18. Diagram of a transverse section through the posterior end of the abdomen. *d.*, dorsal epithelium; *g.*, ventral ciliated groove; *n.*, ventral nerve cord; *r.*, rectum; *s.*, swollen neuropodium with chaetae; *u.*, notopodium with uncinus.

This paper is an account of part of the work carried out during the period of tenure of a grant from the Department of Scientific and Industrial Research. The author wishes to thank Prof. A. D. Hobson for the facilities and help given while working in his department, and also Mr N. Tebble for suggestions at the manuscript stage.

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SUMMARY

The attached tube of *P. triqueter* is described with special reference to the calcareous grating which is usually found inside the posterior end of damaged tubes. An explanation of the morphology of the tube, and of the calcareous grating, is attempted with reference to an account of the mode of deposition characteristic of the worm.

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REQUIREMENT FOR THIAMINE AMONG SOME MARINE AND SUPRA-LITTORAL PROTISTA

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(Text-fig. 1)

It has been known for some years that a need for an exogenous source of thiamine exists among algal flagellates (Lwoff & Lederer, 1935; Provasoli & Pintner, 1953) and indeed the requirement coupled with one for B_{12} is quoted as characterizing auxotrophic algae (Provasoli, McLaughlin & Droop, 1957). Nevertheless, published data for marine species concerning thiamine are limited to a recent paper by J. J. A. McLaughlin (1958) which establishes an absolute requirement for thiamine on the part of *Prymnesium parvum*. The reason for this is partly due to interest in thiamine taking second place to interest in vitamin B_{12} and partly to the fact that thiamine-free media are not easily prepared, so that the requirement for the vitamin appeared as stimulatory rather than absolute (Droop, 1953*a*, 1957).

The following is an attempt to remedy this state of affairs as regards a few species maintained in bacteria-free culture at Millport.

METHODS

The strains used

In the first instance B_{12} requiring strains were chosen for the study, then two known to have no B_{12} requirement were added and finally some dinoflagellates on which there were no data (Table 1).

Culture media

Basal media were as follows: for *Skeletonema*, S36 with thiamine omitted (Droop, 1955*b*); for *Hemiselmis*, S46 with thiamine omitted (Provasoli *et al.* 1957); and for the remainder, excepting *Oxyrrhis*, S50 with thiamine omitted. S50 is given in full in Table 2. It differs from my previously published media chiefly in the composition of the trace metal mixture and the replacement of the pH buffer tris(hydroxymethyl)aminomethane by glycylglycine (which is very much less toxic to some species than TRIS) and glycine (which increases the buffering of the medium in the region of pH 9–10).

The media for *Oxyrrhis* are to be discussed fully in a paper on the nutrition of this species shortly to be published. Thiamine data are included here for the sake of completeness.

Both stocks and experimental cultures were kept in Pyrex test-tubes 15 × 150 mm. Oxo aluminium test-tube caps were employed to begin with, but were replaced by cotton wool when it was realized that contamination from this source was certainly less than 10 mμg/l.

TABLE 1. STRAINS USED

(Millport reference numbers in parentheses)

Chrysophyceae		
Monochrysis lutheri	(60)	Droop, 1953*a*, 1954
Prymnesium parvum Carter	(65)	Droop, 1954
Syracosphaera elongata	(62)	Droop, 1954, 1955*a*
Microglena arenicola	(72)	Droop, 1955*a*, 1957
Cryptophyceae		
Hemiselmis virescens	(64)	Droop, 1955*a*, 1957
Chlorophyceae		
Nannochloris oculata	(66)	Droop, 1955*a*
Bacillariophyceae		
Skeletonema costatum (Grev.) Cleve	(73)	Plymouth strain, Droop, 1955*b*
Phaeodactylum tricornutum Bohlin (syn. *Nitzschia closterium* f. *minutissima* (W. Smith) Allen & Nelson)	(14)	Finnish strain, Droop, 1953*a*
Dinophyceae		
Glenodinium foliaceum Stein	(47)	Finnish strain, Droop, 1953*a*
Peridinium trochoideum (Stein) Lemm.	(88)	From the Clyde
Oxyrrhis marina Dujardin	(18)	Finnish strain, Droop, 1953*b*

TABLE 2. MEDIUM S50

NaCl	15 g	Br	22 mg	Na_2EDTA	50 mg	Glycylglycine	500 mg
$MgCl_2 6H_2O$	2·5 g	Sr	3·8 mg	Fe	500 μg	Glycine	250 mg
KCl	400 mg	Al	28 μg	Mn	50 μg	KNO_3	100 mg
$CaSO_4 2H_2O$	500 mg	Rb	61 μg	Zn	5·0 μg	K_2HPO_4	10 mg
		Li	6·0 μg	Cu	5·0 μg	B_{12}	100 mμg
		I	20 μg	Co	500 mμg	Thiamine	1·0 mg
				Mo	500 mμg	H_2O to	1·0 l.
						pH adjusted to 8	

Where possible, growth was measured optically, in which case it is expressed as percentage transmission or as OD × 100, $\left(= 100 \log_{10} \frac{100}{\%\ \text{transmission}}\right)$. It was necessary to count in other cases; growth is then expressed as cells per mm^3.

RESULTS

Serial transfer experiments

The concentrations over which growth is a function of thiamine concentration in other micro-organisms is 0–500 mμg/l. (Snell, 1951); and since my stock media contained up to 1 mg/l. of the vitamin, at least two transfers were necessary to reduce the concentration sufficiently in the blanks (cultures receiving no addition of the vitamin). This naturally depended on the size of the inoculum which was, therefore, made as small as possible. With *Monochryses*, *Prymnesium*, *Syracosphaera*, *Microglena*, *Nannochloris*, *Phaeodactylum* and *Skeletonema* the inoculum could be reduced to 0·02 ml., thus allowing a 300-fold reduction at each transfer, so that the blanks of the second should have contained about 10 mμg/l. With *Hemiselmis* and the dinoflagellates, however, an inoculum of 0·2–0·3 ml. was advisable which only allowed a 20-fold reduction, with the consequence that four transfers were necessary to deplete the blanks to the same extent.

Transfers were made from the blanks. To the other cultures the vitamin was added initially in the form of the complete molecule (0·1 mg/l.), but later

TABLE 3. SERIAL TRANSFER EXPERIMENTS WITH THIAMINE

(−, blank; +, with 0·1 mg/l. thiamine. OD × 100.)

Transfer No.	Species													
	Nannochloris		*Phaeodactylum*		*Monochrysis*		*Prymnesium*		*Syracosphaera*		*Microglena*		*Hemiselmis*	
	−	+	−	+	−	+	−	+	−	+	−	+	−	+
1	66	68	57	70	122	110	80	92	44	41	35	52	32	70
2	92	92	89	100	30	140	0	80	16	39	11	48	7	110
3	110	100	92	89	4	150	2	80	27	59	22	43	2	105

TABLE 4. *SKELETONEMA COSTATUM*, SERIAL TRANSFER EXPERIMENTS

(OD × 100. standard errors in parentheses, $n = 5$.)

Transfer no.	Treatment		
	Blank	1·0 mg/l. Na_2S9H_2O	0·1 mg/l. thiamine
2	27 (5·4)	21 (9·0)	40 (3·7)
3	39 (1·0)	39 (0·8)	39 (0·7)
4	0·4 (0·4)	29 (1·25)	21 (3·4)
5	21 (0·7)	30 (1·05)	32 (1·1)

TABLE 5. DINOFLAGELLATE SERIAL TRANSFER EXPERIMENTS

(Numbers per mm^3.)

Transfer no.	Treatment				
	Blank	Thiamine	Thiazole + pyrimidine	Thiazole	Pyrimidine
		Oxyrrhis marina			
1	32	119	113	125	25
2	8	73	129	72	5
		Peridinium trochoideum			
2	9	8	13	13	7
3	6	8	6	13	3
4	9	7	8	6	9
		Glenodinium foliaceum			
2	34	26	27	30	24
3	20	28	27	33	28
4	—	33	39	28	31
5	28	21	26	26	25

the molecule was 'split' into its components, 4-methyl-5-β-hydroxyethyl-thiazole and 2-methyl-4-amino-5-aminomethyl-pyrimidine, and the following treatments given: (*a*) blank, (*b*) complete molecule, (*c*) thiazole and pyrimidine in equal amounts, (*d*) thiazole alone, and (*e*) pyrimidine alone.

The results of the transfer experiments are shown in Tables 3–5.

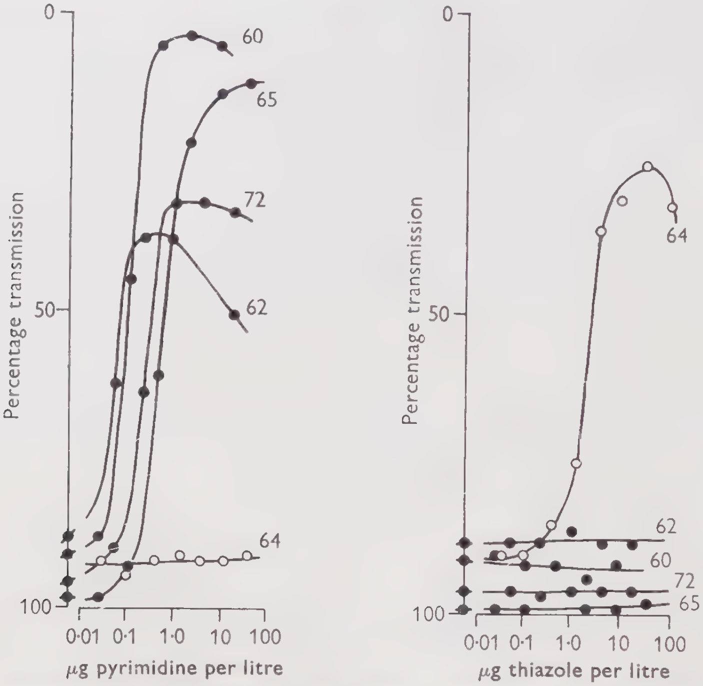

Fig. 1. Response to pyrimidine (left hand) and thiazole (right hand). 60, *Monochrysis lutheri*; 62, *Syracosphaera elongata*; 64, *Hemiselmis virescens*; 65, *Prymnesium parvum*; 72, *Microglena arenicola*.

Dose/response experiments

Where time and the material allowed the transfer experiments were confirmed by dose/response experiments in which was measured yield in response to graded doses of thiamine and its component parts. Response curves for the chrysomonads and *Hemiselmis* to pyrimidine and to thiazole are set out in Fig. 1.

DISCUSSION

Table 6 summarizes the results and includes data of other requirements, notably that for B_{12}. Previous reports regarding *Phaeodactylum*, *Nannochloris* and *Skeletonema* are confirmed (Peach & Drummond, 1942; Hutner, 1948; Droop, 1955*a*, *b*). All but two of the species under discussion in this paper are of littoral or supra-littoral origin, the pelagic strains being *Skcletonema* and *Peridinium trochoideum*: neither had an absolute requirement for thiamine.

Skeletonema has been rather difficult to work with in defined media, because the vigour of cultures seems to depend to a large extent on such factors as pH, oxygen tension and the state of the inoculum—whether, for instance, it is in the exponential or stationary phase of growth, and its relation to the auxospore cycle. Under favourable conditions growth is quite independent of thiamine, and at such times thiamine does happen to be beneficial (as in the fourth transfer, Table 4) it can always be replaced by inorganic sulphide; consequently, it is unlikely that the vitamin is functioning here in this same way as in other organisms. Incidentally, neither *Hemiselmis* nor *Oxyrrhis* could utilize inorganic sulphide in place of thiazole. The significance of sulphur-containing compounds in diatom growth has been discussed by Harvey (1939, 1955); Lewin (1954) and Droop (1957)

TABLE 6. SUMMARY OF RESULTS, WITH DATA ON OTHER HETEROTROPHIC TENDENCIES

	Requirement for thiamine	Portion of vitamin required	Requirement for vitamin B_{12}	Other heterotrophic tendencies
Chrysophyceae				
Monochrysis	+	Pyrimidine	+	Can utilize some amino acids as N source (Droop, 1955*a*)
Prymnesium				
Syracosphaera				
Microglena				
Chlorophyceae	–	–	–	Can utilize some amino acids as N source (Droop, 1955*a*)
Nannochloris				
Bacillariophyceae				
Phaeodactylum				
Skeletonema	–	–	+	Organic compounds sometimes stimulatory in an unspecific way (Droop, 1957)
Cryptophyceae				
Hemiselmis	+	Thiazole	+	Amino N obligatory (Droop, 1957)
Dinophyceae				
Oxyrrhis			?	Amino N obligatory; acetate as C source; phagotrophic in Nature; other B vitamins (unpublished data)
Glenodinium	–	–	+	?
Peridinium				

The results appertaining to two photosynthetic dinoflagellates, *Peridinium trochoideum* and *Glenodinium foliaceum* (Table 5) are not completely satisfactory owing to the lightness of growth normally obtained with these two species. It is noted, therefore, simply that no requirement was demonstrable even after four transfers. On the other hand, a requirement for vitamin B_{12} became apparent on the first transfer and it was not possible to carry either species through more than two transfers in B_{12}-free media.

The four chrysomonads all responded to the pyrimidine half of the vitamin, whereas *Hemiselmis* and *Oxyrrhis* responded to the thiazole half (Fig. 1).

Possibly this represents a phyletic difference between the Chrysophyta and Pyrrophyta, though it is early to tell. With the exception of *Oxyrrhis*, all the species which proved to need thiamine had already been found to have a requirement for vitamin B_{12} (Droop, 1957), but the reverse was not the case.

The Chrysomonads are quite indifferent to the presence or absence of thiazole in the medium, so their response to pyrimidine can be regarded as simple. *Hemiselmis* and *Oxyrrhis*, on the other hand, appear to obtain some benefit from pyrimidine: pyrimidine does not support growth and, in the presence of thiazole, affects yield only slightly, nevertheless, *Hemiselmis* bleaches more quickly in its absence and *Oxyrrhis* fails to produce the fine pink colour characteristic of heavy healthy cultures.

It is not possible to arrive at more than a rough estimate of the magnitude of the thiamine requirement from dose/response curves based on optical measurements (Fig. 1). Furthermore, the basal medium S 50 was developed to suit *Monochrysis* and was not necessarily that most suitable for the other species. Half-maximal growth of the pyrimidine-requiring organisms appeared to be given by 100–300 mμg/l. pyrimidine or thiamine; half-maximal growth of both *Oxyrrhis* and *Hemiselmis* (requiring thiazole) by ten times as much, i.e. 2000 mμg/l., thiazole or thiamine. Such a difference in magnitude in thiamine requirement between pyrimidine and thiazole-requiring species has rather interesting implications should it prove to be real and of general occurrence. It suggests either that thiamine has a different function according to whether the requirement is met by one-half of the vitamin or by the other, or, alternatively, that the functions of pyrimidine and thiazole are divorced from each other and that the function of thiamine here is merely to supply either thiazole or pyrimidine as the case may be. Which alternative is biochemically less improbable it is difficult to say. On the other hand, it is possible that thiazole is merely more labile than pyrimidine.

SUMMARY

The requirement for thiamine was examined in eleven marine protists of littoral, supra-littoral or neritic origin. Six were found to have an absolute requirement for the vitamin.

The thiamine-requiring Chrysophyta responded to the pyrimidine half of the vitamin, whereas the two thiamine-requiring Pyrrophyta responded to the thiazole half.

All the species requiring thiamine were auxotrophic with respect to at least one other factor (usually vitamin B_{12}).

For half-maximal growth, species responding to pyrimidine required 100–300 mμg/l. pyrimidine or thiamine and species responding to thiazole 2000 mμg/l. thiazole or thiamine.

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BIOLOGICAL DIFFERENCES BETWEEN SEA WATERS: EXPERIMENTS IN 1954 AND 1955

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The experiments described and discussed below were made during the 1954 and 1955 breedings seasons of *Echinus esculentus*. They were a continuation of those made in previous years (Wilson, 1951; Wilson & Armstrong, 1952, 1954) and were designed to obtain more information concerning the factors responsible for biological differences between sea waters. In spite of the indefiniteness of many results it is considered desirable to publish them now because it does not seem likely that they can be supplemented in the near future, and because they do contain information which may influence further work in this field.

We should again like to express our thanks to the Marine Station at Millport, especially to Mr E. Latham, for co-operating with us in the collection of water samples.

THE EXPERIMENTS

Methods did not differ in any material respect from those already described in our earlier papers, with the exception of the sea water extracts used in Expt. 8. In making these sea water was passed slowly through a Berkefeld candle and then through a column of active carbon (copper-free). The carbon was then washed with a little distilled water, dried at room temperature, and extracted with successive small portions of cold pyridine until no further coloured material was removed. The extracts were combined and evaporated *in vacuo* at room temperature; waxy brown solids were obtained which dispersed readily in water. A 'blank' extract was also made from the untreated carbon. 18·0 l. of E 1 water gave 0·0547 g of material which was dispersed in 18 ml. water. 18·5 l. of Clyde water gave 0·0441 g, which was dispersed in 18·5 ml. water. The blank extract was dispersed in 18 ml. water. For the experiments 8·0 ml. of these concentrates were added to 2 l. of artificial sea water.

EXPERIMENT I

Celtic water collected 12. iii. 54. Ship: R.V. *Sarsia* with Dr L. H. N. Cooper, position 51° 11′ N., 06° 13′ W. Strained through 200-mesh bolting-silk into carboy. Also two samples filtered through sterile Doulton candles into sterile Winchesters. A sample of bottom water, 118 m, and a sample of bottom mud, 123 m, were collected with a chromium-plated Nansen Petterson water bottle. The mud with some water was kept for 5 days in a glass container and then filtered on a fine paper (previously well washed with sea water from the same station). The mud filtered off was dried and weighed; it amounted to 7·2 g dry matter per litre of sea water. The filtrate is referred to below as 'mud extract'.

E 1 *water* collected 12. iii. 54. Ship: M.F.V. *Sula* with F.A.J.A. Strained through 200-mesh bolting-silk into carboy. Also two samples filtered through sterile Doulton candles into sterile Winchesters.

Clyde water collected 12. iii. 54. Small boat with Mr E. Latham. Strained through 200-mesh bolting-silk into carboy. Also two samples filtered through sterile Doulton candles into sterile Winchesters.

The waters collected in the carboys were further filtered through Doulton candles immediately before use.

The sea-urchins were trawled on 17 March and kept overnight under circulation, the fertilization being made at 4 p.m. on 18 March. Many urchins were opened, almost all had well-filled gonads, few seemed to have spawned. It was with some difficulty that a female with ripe ova in apparently good condition was found. The fertilization was made in a mixture of all three natural waters and the eggs were immediately divided equally between three beakers, and each part washed with six changes of one of the natural waters at room temperature (approx. 15° C—throughout the experiment the room temperature remained very uniform). The fertilization membranes were well elevated but $1\frac{1}{4}$ h after fertilization the cytoplasm of all the eggs examined (from all three waters) assumed an irregular indented shape, rounding up again shortly before first cleavage. The irregular shapes assumed were varied and striking; such well-marked irregularities were not observed in any of the fertilizations for the later experiments in 1954. After first cleavage eggs were distributed in approximately equal numbers to the various dishes comprising the undermentioned sets. Eggs in Sets IV–VI were further washed two changes in their respective presumed sterile waters and it was calculated that only about 0·3 % of the carboy-collected waters, in which the eggs were first washed, would remain in these dishes. Eggs supplied to Sets XIII–XV were taken in equal numbers from all three natural waters in which they had been washed since fertilization, and were further washed in three changes of artificial water before being distributed to the dishes of these sets. Sets XVI and XVII were supplied with eggs washed in E 1 water.

Set		No. of dishes comprising set
I	Celtic water from carboy	4
II	E 1 water from carboy	4
III	Clyde water from carboy	4
IV *a*	Celtic water from Winchester A (proved sterile)	2
IV *b*	Celtic water from Winchester B (proved sterile)	2
V *a*	E 1 water from Winchester C (proved sterile)	2
V *b*	E 1 water from Winchester D (not sterile)	2
VI *a*	Clyde water from Winchester E (not sterile)	2
VI *b*	Clyde water from Winchester F (not sterile)	2
VII	Celtic water + 10% Celtic mud 'extract'	4
VIII	E 1 water + 10% Celtic mud 'extract'	4
IX	Clyde water + 10% Celtic mud 'extract'	4
X	Celtic water + 10% Celtic bottom water	4
XI	E 1 water + 10% Celtic bottom water	4
XII	Clyde water + 10% Celtic bottom water	4
XIII	Artificial sea water	3
XIV	Artificial sea water + 10% Celtic mud 'extract'	3
XV	Artificial sea water + 10% Celtic bottom water	3
XVI	Celtic mud 'extract' undiluted	1
XVII	Celtic bottom water undiluted	1

The fertilization was not quite 100% successful and therefore in every dish there were on the bottom a small number of unfertilized eggs which eventually decayed. The experiment was continued for 7 days. Until 22 March the larvae in all the dishes of any one set were identical as regards swimming vigour, structure and range of variation in structure of the plutei (a proportion were mis-shapen), and in the numbers dead. After that date some variation within a set was observed in some instances, but mostly such differences as did occur were between the larvae of one set and those of another.

The carboy waters from the three localities (Sets I–III) gave almost identical rearings. Not until 22 March was there any definite distinction; on that day the larvae in the Celtic water were rapidly failing in all four dishes. They had begun to fail also in the E 1 and Clyde waters but not to the same extent. The difference was more marked the next day, although by that time there were differences between dishes within a set. The Clyde water proved to be slightly better for the larvae in these later stages than the E 1 water. The results with the dishes were confirmed by comparing the larvae left in the wash beakers.

Of the water samples filtered through Doulton candles at the time of collection only three proved to be sterile when the Winchesters were opened just before use. Two of these were from the Celtic sea, the other from E 1. In these waters (Sets IV *a* and *b*, V *a*) the larvae did not do quite so well as in the carboy waters (Sets I–III) but the difference was not great, especially in the E 1 water (Set V *a*), where the larvae were only just perceptibly inferior to those in Set II. The three remaining water samples filtered at the time of collection were not sterile. In that from E 1 (Set V *b*) the larvae were at all times closely similar to those in Set II. In the Clyde water (Sets VI *a* and *b*) the larvae were inferior to those in the carboy water (Set III), markedly so in Set VI *a*, slightly so in Set VI *b*. These results with both sterile and non-sterile water collected through candles are not entirely satisfactory. The candles were delivered by the maker only the day before the apparatus had to be got ready for use and it is possible that the soaking and washing they were given was insufficiently prolonged to ensure the removal of all soluble substances.

The addition of approximately 10% Celtic bottom water to the three natural sea waters and to the artificial (Sets X–XII, and XV) had little or no effect on the larvae reared in them for the first few days, but on and after 22 March a slight though definite improvement was to be seen, the larvae in the waters with the addition failing less rapidly than in those without. On the other hand, the addition of Celtic mud 'extract' while at first slightly stimulating in that on the third day, in all waters to which it had been added (Sets VII–IX and XIV), the larvae were swimming a little more vigorously towards the surface than in the waters without, in the later stages the larvae failed a little more rapidly than in these latter. In the dishes of undiluted Celtic mud 'extract' and Celtic bottom water (Sets XVI and XVII) the larvae were in poor condition even on the second day and in the mud 'extract' they were quickly killed. There is thus shown a graded series: the undiluted mud 'extract' poisonous; the undiluted bottom water harmful but not quite poisonous, the larvae survived in it abnormally for several days; the addition of a small volume of mud 'extract' at first slightly stimulative, but the larvae failed more quickly than in water without; the addition of a small portion of a small volume of bottom water without at first apparent effect, but the larvae survived a little longer than in the waters without.

EXPERIMENT 2

The difficulty experienced in obtaining good ova for Expt. 1 led to a fresh batch of sea-urchins being trawled on 19 March 1954 and a new fertilization made as soon as they were landed. As before, many ovaries contained irregular fragments of ova.

A female which looked better than the rest was chosen. After fertilization the eggs were divided into three equal portions and washed in the three kinds of sea water. A sample of the eggs was repeatedly examined until first cleavage, but this time the cytoplasm did not indent irregularly. The only tests made were of the three waters collected in carboys and filtered through Doulton candles just before use.

Set		No. of dishes comprising set
I	Celtic water from carboy	3
II	E 1 water from carboy	3
III	Clyde water from carboy	3

The results were closely similar to those obtained with Sets I–III in Expt. 1. The plutei were on the whole more even in structure with fewer mis-shapen individuals than in Expt. 1, but they never attained the fine development of plutei in the best rearings in the Celtic and Clyde waters of 1949, 1950 and 1952. Five days after fertilization most of them went to the bottom and lost their arms. As in Expt. 1 the larvae in all three waters were closely similar, but they failed a little earlier in the Celtic water and at the end were a little better in the Clyde water than in the other two, though the difference was no more than perceptible.

EXPERIMENT 3

E 1 surface water collected 29. iii. 54. Ship: R.V. *Sarsia* with F.A.J.A. Strained through 200-mesh bolting-silk into carboy. Also one sample filtered through sterile Doulton candle into sterile Winchester. A sample of water at a depth of 70 m was collected with a chromium-plated Nansen Petterson water bottle after this bottle had been used for the collection of several samples for hydrographical purposes.

Shortly before use the water collected in the carboy was filtered through a Doulton candle. The water which had passed through a Doulton candle at the time of collection proved to be sterile when the Winchester was opened on 1 April immediately before the experiment began. The carboy water before filtration gave, on the other hand, a heavy growth of mixed organisms when bacteriologically tested at the same time.

The sea-urchins were trawled on 1 April and used immediately. Of the many opened only a few were spent. In general the ovaries seemed to be in better condition than a fortnight previously and there was less difficulty in selecting a female for spawning. Immediately after the fertilization was made in E 1 surface water the eggs were divided into four equal quantities and each quantity washed with six changes of either E 1 surface water, E 1 sterile surface water, E 1 deep water from 70 m, or four changes of artificial sea water. Before first cleavage took place the cytoplasm of some of the eggs became irregular in outline and slightly indented (but not as markedly as in Expt. 1), rounding up again before cleavage. After first cleavage equal quantities of eggs were distributed as follows. There were four dishes in each set, two in each half-set in Series IV and V.

Set		Eggs washed between fertilization and first cleavage in
I	E 1 surface water (carboy)	E 1 surface water
II	Sterile E 1 surface water	Sterile E 1 surface water
III	E 1 deep water (from 70 m)	E 1 deep water
IV	E 1 surface water + 50% deep water	(*a*) E 1 surface water (*b*) E 1 deep water
V	E 1 surface water + 10% deep water	(*a*) E 1 surface water (*b*) E 1 deep water
VI	Artificial sea water	Artificial sea water
VII	Artificial sea water + 10% E 1 surface water	Artificial sea water
VIII	Artificial sea water + 10% E 1 deep water	Artificial sea water

The duration of the experiment was about 1 week. There was little difference in the condition of the larvae between Sets I and II, the water sterile until the experiment began being slightly less favourable than the carboy water. By this time the Doulton candle had been well washed before use.

The deep water (Set III) proved much less favourable than either of the surface waters (Sets I and II); from an early stage the larvae in it were mainly abnormal and in 2 or 3 days many were dead or dying. The mixtures of surface and deep water are of particular interest because the results with them were strikingly different according to whether the eggs were washed between fertilization and first cleavage in surface water or in deep water. With those washed in surface water (Sets IV*a* and V*a*) the results were similar to those with surface water alone (Sets I and II) being only a little less favourable in the 50% mixture. But with eggs washed in deep water (Sets IV*b* and V*b*) the larvae were decidedly inferior to surface water Sets I and II and only a little better at first than those in deep water alone (Set III). It will be realized that these results with deep water (which are similar in some respects to those obtained with the bottom water and mud used in Expt. 1, but in that experiment eggs did not come into contact with the bottom water until after first cleavage) could be explained by faulty technique if the beaker in which the eggs washed with the deep water had contaminated that water. The beaker had, however, been well cleaned with hot strong sulphuric acid before use, but it was not a new beaker. It could also be explained if the chromium-plated water bottle had affected the water.

The larvae in artificial sea water (Set VI) were poor compared with those in surface water (Sets I and II) although better than those in deep water (Set III). An addition of 10% surface water affected a slight improvement, while the addition of 10% deep water had little or no effect. These were the poorest larvae so far reared in artificial sea water in the course of these experiments; it should perhaps be noted that in earlier experiments the eggs were not washed into artificial sea water quite so soon after fertilization as they were here.

EXPERIMENT 4

*E*1 *surface water* collected 12. iv. 54. Ship: M.F.V. *Sula* with F.A.J.A. Strained through 200-mesh bolting-silk into carboy. The presence of *Phaeocystis* was noted. Also samples of both surface and deep water from 70 m collected in plastic-lined water bottle, and stored in Winchesters.

Before use all waters were filtered. That from the carboy was passed through a Berkefeld candle and was used for making the fertilization. The waters from the Winchesters were aerated after passing through the Doulton candles and were used for washing the eggs and for filling the experimental dishes. Thus in this experiment the waters compared had both been collected in the same way. Moreover, the use of the plastic water bottle instead of the chromium plated one used for the experiment would resolve doubts concerning the latter. The beakers used for washing the eggs were new ones, thoroughly cleaned in hot strong sulphuric acid.

The sea-urchins were trawled on 13 April and used at once. The ovaries seemed to be in better condition than last time and almost 100% after fertilization had well-elevated membranes and even cleavage. No shrinking of the cytoplasm was noted between fertilization and first cleavage. The eggs were divided into two equal parts immediately after fertilization and one lot washed with surface water and the other with deep water, six changes of each. After first cleavage approximately equal numbers of eggs were distributed to the following sets of dishes, four dishes to each set.

Set		Eggs washed with
I	E 1 surface water	E 1 surface water
II	E 1 surface water	E 1 deep water
III	E 1 deep water	E 1 surface water
IV	E 1 deep water	E 1 deep water
V	Mixture: 50% surface 50% deep water	E 1 surface water
VI	Mixture: 50% surface 50% deep water	E 1 deep water

In spite of the apparent excellence of the fertilization a considerable proportion of the eggs died during late cleavage stages before the formation of the blastula, and many blastulae were abnormal, only a small proportion appearing normal. That this was not due to the plastic water bottle in which the experimental waters were collected is shown by the fact that the eggs and blastulae were identical in a control Berkefeld filtered water from the carboy, these eggs not having come into contact with water from the plastic water bottle at all. Unfortunately, in this experiment no water other than that from E 1 was available for comparison, and no fertilization in this water of another batch of eggs and sperm. In spite of these aspects being uncontrolled and of the relatively poor condition of the best larvae the experiment gave a striking result. For the first 2 days after fertilization all dishes in all sets were alike as regards their larval content. The larvae if not already dead, were slow to swim up, most of them remaining on the bottom even as late as the gastrula stage. The majority showed some abnormality, the gastrulae on the whole being small and shrunken. A day later (16 April) almost all those in deep water (Sets III and IV) were dead and so were those which had been washed in deep water between fertilization and first cleavage, although then put into surface water (Set II—it must be noted that this water would contain about 7·5 % deep water, added to it with the larvae). Only those larvae which had never at any time been in contact with deep water (Set I) were still mainly alive and swimming well, even more vigorously than before and on the whole a little more normal-looking. Sets V and VI with mixed water, whatever water the eggs had been washed with, were in much poorer condition than these, but strangely not quite so bad as Set II with much less deep water mixed with it. This would be understandable for Set V (eggs washed in surface water) if the eggs are specially susceptible to damage between fertilization and first cleavage, and were damaged by the deep water, but would not explain why Set VI (eggs washed in deep water) was better than Set II which contained a much smaller proportion of deep water.

The experiment was not seen again until 20 March. Conditions on that day neither added nor subtracted anything from the result.

EXPERIMENT 5

Clyde water collected 26. iv. 54 in Largs Channel, tide falling. Small boat with Mr E. Latham. Strained into carboy through 200-mesh bolting-silk, which was discoloured brown by diatoms.

Artificial sea water. The salts used were the same as in 1953 except for the CaCl$_2$ which was a new batch.

A more elaborate experiment had been planned. Unfortunately, stormy weather prevented the collection of natural sea waters at Plymouth and it was only possible to compare the Clyde water with artificial sea water and to investigate the effect of washing the eggs between fertilization and first cleavage in both waters. The Clyde water was passed through a Berkefeld filter-candle before use.

The sea-urchins were trawled on 28 April and used at once; most of them were spent. The eggs selected gave an almost 100 % fertilization with well-elevated fertilization membranes. Less than 15 min after adding the sperm the eggs were divided into one of the two waters. Each water was changed six times and after first cleavage the eggs were distributed to the following sets of four dishes each. It should be noted that each dish would receive with the eggs a little of the water in which they had been washed. It was calculated that this water would amount to about 8% of the total volume of water in the dish.

Set		Eggs washed with
I	Clyde water	Clyde water
II	Clyde water	Artificial sea water
III	Artificial sea water	Clyde water
IV	Artificial sea water	Artificial sea water
V	Mixture in equal proportions of Clyde and artificial sea waters	Clyde water
VI	Mixture in equal proportions of Clyde and artificial sea waters	Artificial sea water

In spite of the apparent excellence of the fertilization the blastulae were slow to swim up in all dishes and the majority took an unusually long time to reach the surface and most were beginning to gastrulate before they did so. They developed better in the Clyde water than in the artificial sea water, their development in the mixture being nearly as good, though not quite, as in the Clyde water alone. The kind of water the eggs were washed in between fertilization and first cleavage made no significant difference. In all waters plutei became abnormal by the third or fourth day, earlier and to a greater extent in the artificial sea water than in that from the Clyde water. It has been noted that the latter had a heavy growth of diatoms in it at the time of collection, but as no comparison was made with any other natural sea water it is impossible to decide whether the relatively poor conditions of the larvae at all times in this water is to be attributed to its constitution or to the condition of the eggs or sperm.

EXPERIMENT 6

Clyde water collected 4. v. 54, in Largs Channel approx. an hour before high tide. Small boat with Mr E. Latham. Strained into two Winchesters through 200-mesh bolting-silk; hardly any diatoms present. At the same time two further samples (A and B) were collected through Doulton candles into evacuated sterile Winchesters using the method described in Wilson & Armstrong, 1954, pp. 348–9. Particulars of artificial water as for Expt. V.

Continuing bad weather prevented the collection of natural sea water at Plymouth. This experiment embodied two separate investigations: (1) a further attempt to compare sea water strained only through bolting-silk at the time of collection into clean but non-sterile glass containers, with sea water collected at the same time through Doulton candles into sterile containers and tested for sterility just before use; (2) to investigate the effect of adding a quantity of sea water from which a variety of thick growths of phytoplankton organisms had filtered out. We are indebted to Dr Mary Parke for supplying a number of thick cultures of various diatoms and flagellates for this purpose. These cultures were filtered through Doulton candles. Dr Parke also supplied the sample of unused sterile culture medium ('Erdschrieber') for our controls.

We are indebted to Dr C. H. Jellard, Public Health Laboratory Service, for bacteriological tests of the Clyde waters A and B, these being sampled bacteriologically on 6. v. 54 shortly before the experiment began. At the same time bacteriological samples were taken from the Winchesters which contained the water filtered only through bolting-silk. These latter samples gave heavy growths of a number of different organisms, the waters A and B gave no visible growths for 2 days, but later each gave some growth of a single organism, in each case a slow-growing Gram-negative bacillus, a different one in A from that in B. The control plates which had not been inoculated were sterile.

The sea-urchins were trawled on 6. v. 54. Almost all were spent, or partially spent. Only one female in reasonably good condition was obtained and a few males. A fertilization was made in Clyde water filtered from the carboy which supplied Expt. V. The eggs were immediately divided into four equal quantities; one washed with six changes of new Clyde water from the Winchesters (filtered through Doulton candles in the laboratory), two other quantities each with six changes of Clyde waters A and B, and one quantity with six changes of artificial sea water. The temperatures of all waters were adjusted to room temperature before use.

Almost all eggs fertilized and showed good membrane elevation; a few protruded cytoplasm through the membrane after about ½ h. The remainder eventually produced mainly normal blastulae even in size and appearance. At about the time of first cleavage equal quantities of eggs were distributed, from the appropriate washing waters, to the following sets of dishes.

Set		No. of dishes in set
I	Clyde sea water not sterile	4
II	Clyde sea water (A) proved almost sterile until used	4
III	Clyde sea water (B) proved almost sterile until used	4
IV	Clyde sea water (as I) + 10% culture medium (control)	4
V	Clyde sea water (as I) + 50% culture medium (control)	2
VI	Clyde sea water (as I) + 10% culture filtrate	4
VII	Clyde sea water (as I) + 50% culture filtrate	2
VIII	Artificial sea water	4
IX	Artificial sea water + 10% culture medium (control)	4
X	Artificial sea water + 50% culture medium (control)	2
XI	Artificial sea water + 10% culture filtrate	4
XII	Artificial sea water + 50% culture filtrate	2

For the first 2 days there were no observable differences between any of the dishes, the larvae swam up strongly in them all. The subsequent development of Sets I, II and III will be considered first. Three days after fertilization there was a slight tendency for the larvae in the dishes of Set II to concentrate at the surface more than in any other set, but this was not observed the next day though it re-occurred on the sixth day. It must be emphasized, however, that this tendency was never more than barely perceptible. There were no structural differences between the plutei of Sets I, II and III at any stage. They were in fine normal condition on the fifth day, but on the sixth the living tissues of the arms shrank down the supporting rods, which broke away leaving the larvae armless. The following day they were very abnormal in all three sets and many were dead.

The addition of culture medium and culture filtrate to the Clyde water (Sets IV to VII) made little difference. Such differences as were observed indicated that culture medium, when present in fair proportion as in Set V, was on the whole disadvantageous but in lower concentration (Set IV) was very slightly beneficial. The culture filtrate had even less effect.

In the artificial sea water (Set VIII) many of the larvae, which in the early stages swam up strongly, sank to the bottom of the dish a little earlier than in the natural sea waters in Sets I–III, but they did not degenerate quite so quickly as did those in the natural sea waters, although on the whole there was little difference. The addition of culture medium made only a very slight difference, being on the whole disadvantageous in quantity (Set X) and very slightly favourable in smaller amount (Set IX). Culture filtrate made little or no difference.

EXPERIMENT 7

E I *surface water* collected 15. iii. 55. Ship: M.F.V. *Sula* with F.A.J.A. Dipped up with wooden bucket and strained through 200-mesh bolting-silk into glass carboy. Plankton thicker than usual.

E I *bottom water* (70 m) collected same time as surface water using Perspex-lined water bottle. Stored in glass Winchester but not filtered through bolting-silk.

Echinus *ground bottom water* (52 m) collected same day as E I bottom water and in same way. This ground is situated Eddystone N. 160°, Rame Head N. 55° E.

Clyde water (*surface*) collected 14. iii. 55, in Largs Channel. Small boat with Mr E. Latham. Strained through 200-mesh bolting-silk into glass carboy.

Artificial sea water. All waters were filtered in the laboratory through Berkefeld filter candles not more than 1 day before use.

The purpose of this experiment was to compare again E I and Clyde surface waters which of recent years had not been as strikingly different in their properties as they had been several years ago when the first experiments were made. It also was to investigate further the 1954 results in which it appeared that there was a difference between the surface and bottom water at E I, and that the development of the *Echinus* eggs was affected by the water in which the eggs were washed between fertilization and first cleavage (Expts. 3 and 4). As the bottom water at E I had apparently been unfavourable to the development of the eggs it was decided to test bottom water from the *Echinus* grounds themselves. These are situated some miles away from E I, (where the bottom is muddy and there are no sea-urchins) and the water bathing these grounds would be that into which the *Echinus* would spawn under natural conditions. It must in this connexion be remembered, however, that the water over the grounds would be changing and would not be static from day to day, and that it was impossible to know whether on the day chosen for taking the water any of the *Echinus* would be spawning naturally. If their spawning is affected by the particular type of water bathing them it is conceivable that only at certain times, when the water is favourable, do they actually shed their eggs and sperms.

The *Echinus* used were trawled on 18 March from beyond the Eddystone and not on the ground from which the *Echinus* bottom water had previously been taken and where *Echinus* is normally abundant. In March and April 1955, perhaps as the result of the severe gales earlier, *Echinus* proved to be almost absent from this ground and they had to be got elsewhere when needed in quantity. This fact was not known when the bottom water was collected. The fertilization was made as soon as the urchins had been brought into the laboratory; the majority of the urchins were immature but a moderately mature female was found. The eggs and sperms were shed into, and the fertilization was made in, artificial sea water. The same urchins were subsequently spawned into, and a control fertilization made, in E I surface water; this was successful but not particularly good. About $\frac{3}{4}$ h after fertilization the cytoplasm of the eggs became irregular in outline (rather as in Expt. 1), rounding up again 40 min later before first cleavage. Seven minutes after the fertilization in artificial sea water had been made the eggs from it were divided into five equal quantities into the five wash waters. Six changes of these were given and after the first cleavage eggs were distributed in equal quantities to the dishes containing the waters to be tested. There were twelve sets of these, three dishes to each set. The arrangement of the experiment will be clear from the headings in Table 1. Thus Set I comprised three dishes each containing 100 ml. of E I surface water and into them equal quantities of eggs were ladled out of the beaker in which they had been washed with E I surface water to free them from the artificial sea water in which the fertilization had been made. Set II likewise comprised three dishes each containing 100 ml. of E 1 surface water, but into these were put equivalent quantities of eggs washed in E 1 bottom water (and with them about 8 ml. of the latter water from which they could not be separated), and so on through the whole series. The condition of the larvae on subsequent days is recorded in Table 1. First, their swimming vigour as shown by the relative numbers at the surface and bottom, and as evenly distributed in mid-water between surface and bottom, and secondly on the fourth day and thereafter the structural appearance of the plutei is briefly described, together with rough estimates of the numbers observed to have died. There were never any differences between the three dishes of any one set; in so far as they differed from those of another set they all differed alike. Not until after 23 March, on which day the experiment was considered to have ended, did any inconsistencies appear between dishes of the same set and then only to a slight extent. These later stages are not recorded for they were concerned only with farther degeneration and death and did not yield any more information about the waters.

There are several interesting features to be noted concerning the results. It was very noticeable that on the morning of the second day the blastulae swam up more strongly in the Clyde water (Sets VI-X) than in any of the others and in the artificial sea water (Set XII) not at all. On the third day they were swimming well in all dishes but most strongly in both the bottom waters (Sets IV, V, X and XI), while the larvae in the Clyde waters which previously had led now flagged a little, there being more on the bottom than in any of the others and slightly fewer at the surface also. This latter fact is not indicated in the Table for the numbers involved were too few to justify recording a lower category than that shown. By the next day the larvae in the Clyde waters had caught up again with the others, and as regards swimming vigour all dishes were equal except those containing artificial sea water (Set XII) where almost all the larvae, which were structurally abnormal, had sunk to the bottom. These larvae never formed normal plutei although retentive of life. On 21 March the larvae of one dish were transferred to Clyde water and one other to artificial sea water and Clyde water in equal proportions. The following 2 days more larvae swam up off the bottom of these dishes than did those in the remaining dish of artificial sea water only (which alone is recorded in Table 2 for 22 and 23 March), but they never showed any indication of developing into plutei. On 21 March it was observed for Sets I–XI that the larvae, which were early plutei beginning to grow their first four arms, were decidedly more advanced in development with longer arms and were healthier looking in the Clyde waters than in any of the others. The larvae in all other natural waters looked much alike, except that possibly those in the *Echinus* ground bottom water (Sets X and XI) were a trifle better than those in the E 1 waters, both surface and bottom.

The next 2 days, 22 and 23 March, showed some marked differences. It became clear that Clyde water (Set VI) was proving a better medium for the larvae than E 1 surface water, and to an extent similar to that which existed a few years ago. It was clear too, that whilst washing the eggs between fertilization and first cleavage had not affected them when the wash water was E 1 surface water (Set VII), it had had an adverse effect when this wash water had been either of the bottom waters (Sets VIII and IX), especially the *Echinus* ground bottom water. This latter water although at first promoting swimming vigour did not long support the larvae in health (Sets X and XI) and finished by being almost worse in this respect than any other natural water. It had also proved bad when used as a wash water for eggs placed subsequently in E 1 surface water (Set III). The E 1 surface water itself, had not, as has been seen, given good plutei at any stage, the eggs which had been washed with E 1 bottom water (Set II) doing slightly better than others washed differently (Sets I and III), whilst the E 1 bottom water itself was slightly better (Sets IV and V) than the surface water from

TABLE 1. EXPERIMENT 7, FERTILIZATION OF *ECHINUS ESCULENTUS* MADE IN ARTIFICIAL SEA WATER ON 18 MARCH 1955

Set	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Water in dishes	E I surface	E I surface	E I surface	E I bottom	E I bottom	Clyde surface	Clyde surface	Clyde surface	Clyde surface	*Echinus* ground bottom	*Echinus* ground bottom	Artificial sea water
Wash water	(E I surface)	(E I bottom)	(*Echinus* ground bottom)	(E I surface)	(E I bottom)	(Clyde surface)	(E I surface)	(E I bottom)	(*Echinus* ground bottom)	(E I surface)	(*Echinus* ground bottom)	(Artificial sea water)
19. iii. 35 (a.m.)	Beginning to swim up	As I	As I	As I	As I	Swimming up strongly	As VI	As VI	As VI	As I	As I	All on bottom
20. iii. 55 *S* *M* *B*	* * * * * * * * * * *	As I	As I	* * * * * * * * * *	As IV	* * * * * * * * * * * *	As VI	As VI	As VI	* * * * * * * * * *	As X	* * * * * * * * * *
21. iii. 55 *S* *M* *B*	* * * * * * * * * * Fairly good, very few abnormal	As I As I	As I As I	As I As I	As I As I	As I Good, very few abnormal	As I As VI	As I As VI	As I As VI	As I As I	As I As I	* * * * * * * + Abnormal
22. iii. 55 *S* *M* *B*	* * * * * * * * + Poor, often abnormal, few dead	* * * * * * * * * * * * A little better than in I, few dead	* * * * * * * * * + Poor, many abnormal, several dead	* * * * * * * * * * * * Moderate, fair number abnormal	As IV As IV	* * * * * * * * * * * Good, very few abnormal, very few dead	As VI As VI	* * * * * * * * * * * Moderate, good number abnormal, several dead	* * * * * * * * * * Fairly good, good number dead	* * * * * * * * * * Some moderate, many abnormal, several dead	* * * * * * * * * + As X	* – * – * * * * * + Very abnormal
23. iii. 55 *S* *M* *B*	o * – * * * * * + Very poor, all abnormal, good number dead	* * * * * * * * + Poor, mostly abnormal, good number dead	o o * * * * * + All abnormal, dead or dying	* – * * * * * * * + Slightly better than II, but very similar	As IV Poor, mostly abnormal, good number dead	* * * * * * * * * * * Moderate, majority becoming abnormal, few dead	As VI As VI	* * * * * * * * + Slightly inferior to VI	* * * * * * * + Very poor, all abnormal, many dead	* – * * * * * * + All abnormal, majority dead	o o * * * * * + Very abnormal, almost all dead	o o * * * * * + Very abnormal, several dead

B = bottom; *M* = mid-water; *S* = surface. o = none; * – = very few; * = few; * * = several; * * * = fair number; * * * * = good number; * * * * * = many; * * * * * + = very many.

the same position. This is the reverse of the 1954 findings with E 1 waters (see p. 335). It must be emphasized that all these differences although definite were slight; they were not of the order of the difference between the Clyde water and all the others.

The results standing out from this experiment are: (1) the superiority of Clyde water over all the others, although the plutei were never as well developed or as long lived in it as had sometimes been the case in earlier experiments. This may have been a reflexion of the quality of the eggs or sperm but this point was not controlled. (2) The inability of the eggs to form normal larvae in the artificial sea water, in contradistinction with those reared in artificial sea water in previous years. It may be noted that the sodium chloride used in making up the artificial sea water came this year from a different batch than before. (3) The fact that every difference in the treatment of the eggs, the water they were washed with and the water they developed in had some influence, however slight, on the structure and health of the larvae developing from the eggs, and these differences affected all dishes of a set equally.

EXPERIMENT 8

Celtic sea surface water collected 9. iv. 55. Position 50° N. 10° W. R.V. *Sarsia* with Mr A. D. Mattacola. Strained through 200-mesh bolting-silk into glass carboy. At the same time two further samples (A and B) were collected through Doulton candles into evacuated sterile Winchesters using the method described in Wilson & Armstrong, 1954, pp. 348–9.

E 1 surface water collected 12. iv. 55. M.F.V. *Sula* with F.A.J.A. Strained through 200-mesh bolting-silk into glass carboy. At the same time two further samples (C and D) were collected through Doulton candles into evacuated sterile Winchesters.

Artificial sea water as used in Expt. 1.

As usual the carboy waters were filtered in the laboratory before use, Doulton candles being used. The waters filtered through similar candles at the time of collection were tested for sterility shortly before use and three of them, two being from E 1 and one from the Celtic sea proved to be sterile. We were again indebted to Dr C. H. Jellard of the Public Health Laboratory Service for making the bacteriological tests.

The urchins were trawled on 14 April and used as soon as they arrived in the laboratory. They had unfortunately proved scarce and there were therefore fewer than usual from which to select. The male and female chosen gave what appeared to be an almost 100% perfect fertilization with well-elevated membranes and perfect first and second cleavage. There was no crumpling of the cytoplasm before cleavage, as occasionally noted previously. The fertilization was made in artificial sea water and immediately divided into ten equal parts, each part in a small beaker, and each part was then washed with four changes of one of the waters to be tested, as recorded below. After first cleavage the eggs were distributed to the following sets of dishes, three dishes to a set. The eggs supplied to Sets XI and XII were washed between fertilization and first cleavage in a water other than that to which they were finally transferred, but were subject to further washing after first cleavage to avoid adding to the water in the dishes even a little of another sort as had been done in some earlier experiments.

In spite of the apparent excellence of the fertilization up to and including the second cleavage at 9 p.m. on the first evening, almost all the eggs died in later cleavage stages and by the next morning relatively few had survived to form normal blastulae. All natural waters (Sets I–VI and XII) were equal in these respects, but in the artificial waters (Sets VII–XI) only about half were dead, the remainder being living blastulae, many obviously abnormal. These never developed into plutei, as in artificial sea water in the previous experiment, and the addition of the extracts made no difference whatever.

Set	Water tested. Except where otherwise stated four washings with the same water were given between fertilization and first cleavage
I	E 1 surface water (carboy)
II	E 1 surface water proved sterile until used (Winchester C)
III	E 1 surface water proved sterile until used (Winchester D)
IV	Celtic sea surface water (carboy)
V	Celtic sea surface water proved sterile until used (Winchester A)
VI	Celtic sea surface water filtered at time of collection but not sterile when tested (Winchester B)
VII	Artificial sea water
VIII	Artificial sea water with added blank extract
IX	Artificial sea water with added E 1 extract
X	Artificial sea water with added Clyde extract
XI	Artificial sea water—eggs in Celtic water between fertilization and first cleavage, then washed with artificial sea water
XII	E 1 surface water (carboy)—eggs in artificial sea water between fertilization and first cleavage, then washed with E 1 surface water

With so few left alive in the natural waters definite results are hardly to be expected. At most it can be said that slightly fewer larvae swam up in the sterile Sets II and III than in Set I, and the same thing can be said of Sets V and VI compared with Set IV, although Set VI was not actually sterile. These differences were very slight. There were no distinctions between Celtic and E 1 waters and the best larvae were poor stunted plutei which never properly grew arms. On the fifth day all those living were very abnormal everywhere and the experiment was abandoned.

There seems little doubt that the fertilized eggs had been incapable of normal development; they did badly in all waters. The experiment is, however, of value in that it indicates that the new type of extract is not poisonous, as had previously been the case with carbon and acetone extractions (Wilson & Armstrong, 1954, pp. 351, 355), and this may in future prove to be a useful method of investigating the main problem. It may be useful, too, to put on record that the method devised for obtaining sterile sea water at the time of collection was again successful, and on this occasion three times out of four.

EXPERIMENT 9

It was impossible to repeat again Expt. 8 the same year on account of the organization needed and the fact that no ship would be available to do the necessary collecting until after the end of the breeding season of *Echinus esculentus*. Indeed, for the time being no more sea-urchins could be got and so recourse was had to using *Sabellaria alveolata*, the larvae of which had previously responded to water differences, though not as readily as *Echinus* larvae (Wilson, 1951, p. 14). It seemed desirable at least to test the Celtic and the E 1 carboy waters used in Expt. 8 to make as sure as possible that the failure of that experiment should be attributed to the condition of the eggs and not to the waters.

On 24 April 1955 *Sabellaria alveolata* was collected at Duckpool, near Bude. On 27 April a fertilization (several males and females) was made in a mixture of the same Berkefeld filtered Celtic and E 1 carboy waters used in Expt. 8. After fertilization the eggs were divided equally into four beakers and each given several changes of one of the following four waters.

Set	
I	Aquarium water (unfiltered)
II	Celtic water
III	E 1 water
IV	Artificial sea water

The following day in each beaker all larvae which had swum to the surface were decanted off from unripe eggs on the bottom, and approximately equal quantities of these larvae distributed to the experimental dishes, three dishes in each set.

Until 30 April no significant differences were observed between any of the sets. On that day the larvae in the artificial sea water, which was an unused quantity remaining over from Expt. 8, were seen to be swimming more slowly than in the other sets, and they were not so well developed or as large as the larvae in the natural sea waters. This difference persisted until the experiment was abandoned a week later, by which time larvae in the artificial sea water were losing spines and many were dead. Throughout this time no differences appeared between the other sets of larvae, they developed well for a time and until checked by lack of food.

From these observations it appears that the Celtic and E 1 carboy waters were almost certainly not responsible for the high mortality in Expt. 8. It is also interesting to note that whereas the *Echinus* eggs in the two previous experiments had in artificial sea water failed to produce anything resembling a normal pluteus, the *Sabellaria* eggs in the same artificial sea water developed into larvae structurally normal so far as could be observed, except that they were smaller than those in the natural waters and swam more slowly.

DISCUSSION

The factor which has hampered our experiments more than any other has been the weather. Much initial planning and much careful preparation has been wasted and several experiments have been largely ruined because storms at critical times have prevented the collection of water samples. Storms also had their effect on the collection of *Echinus*, and as in 1955 gales in the weeks preceding the breeding period may, or so it appears, have an adverse effect on the numbers of sea-urchins present on the local grounds and perhaps even on the maturity of those still left there. It was particularly unfortunate, in this connexion, that, when, as for Expt. 8, a number of bacteria-free samples of water had been successfully collected from two localities the fertilization, at first seemingly excellent, failed to produce good larvae in any water, apparently because the zygotes were incapable of development beyond early cleavage, it being unlikely that the natural waters themselves were to blame (see Expt. 9). In the face of such hindrances by natural forces progress in this type of work is likely to continue to be very slow.

It is perhaps not surprising that bottom and surface waters at the same station should affect the developing eggs differently, for it is normal for such waters to be distinguishable chemically. What is surprising is that bottom water should often have proved harmful, especially when the eggs were exposed to it for only a short period following fertilization. It is at this period that eggs would normally be in bottom water after having been shed. It may be that this effect is due to experimental conditions, such as rapidly substituting one water for another. It can hardly be a temperature effect because all waters used were brought to a uniform temperature. If it is real it is a pointer to the possibility that bottom animals may spawn only when the water in which they are bathed is suitable for development. In one experiment the bottom water was as good as, if not better than the surface water.

These results and those with mud extracts may be linked with the need of the developing echinoderm egg and larva to absorb substances from the surrounding medium (Needham, 1931) and it may be necessary for some of these substances to be absorbed very soon after fertilization. The experiments do not indicate that there is anything in filtrates (which contained some natural sea water) from diatom or flagellate cultures, or from culture medium itself, which can supply those needs.

The most interesting criticisms (Walne, 1956, and private discussions with others) which have arisen in the course of our work concern the possible effects of bacteria on the waters collected during the inevitable storage period between collection and use, and of excessive bacterial growths in the experimental dishes during the course of the experiments*. Moreover, these criticisms seemingly always imply that such bacteria produce harmful effects and it does not appear to have been envisaged that they may sometimes be beneficial, if not essential. In saying this we do not wish to imply that we believe that we have any real evidence that bacteria are beneficial: we approach the problems posed by their presence with completely open minds. None the less we do believe that differing bacterial populations would be no more than another demonstrable difference between disimilar water masses and not necessarily in themselves the basic cause of those differences. For the basic cause we must look even further back, behind the bacteria.

In our experiments we have, as recorded in the preceding pages and in an earlier paper (Wilson & Armstrong, 1954), endeavoured to filter water free from bacteria at the time of collection and to compare larvae reared in such water with those reared in water collected at the same time in the usual manner, using as a filter nothing finer than bolting-silk. A modicum of success has attended these efforts but the results obtained are open to varying interpretation. However, so far as they go they do not support the view that bacteria in the water between collection and use make it less suitable for the larvae reared in it: on the contrary such slight differences as were shown between bacteria-free samples and their corresponding waters collected in the ordinary way (carboy waters) were usually in favour of the latter. It is possible that the slightly unfavourable results obtained with the bacteria-free waters are explicable by contact of the water with the previously heated rubber tubing through which it was passed and from out of which substances may have dissolved; also water stored in a Winchester has a larger surface area of glass in contact with it, volume for volume, than has water in a carboy. In future experiments these details should be equalized on both sides. Even as it was, the difference between the two samples of one kind of water collected in the two ways was always much less than the differences which have often been observed between waters from separate localities.

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* See also Walne, 1958 (this Number, p. 415). Although Walne's latest paper was not seen by us until after this discussion had been written, we feel that it already answers the chief points raised by Walne.

Our bacteria-free waters were, of course, free from bacteria only until they were poured out into the experimental dishes and the eggs put into them. Presumably, at that time they had a smaller population of bacteria than did the corresponding carboy-collected water, although perhaps not so much smaller because this latter water was itself passed through filter-candles not many hours before use. Walne (1956) has recently shown how in vessels each containing 1 l. of sea water twice filtered through filter-candles, and to which were added a few ml. of concentrated *Isochrysis* culture together with several hundred oyster larvae, the concentration of bacteria increased during the first 3 or 4 days of the experiments. Presumably more or less the same thing must have happened in our dishes, but no bacterial counts were made. Certainly if some of the larvae died after a few days the water in the dishes became slightly cloudy, but it is difficult here to distinguish between cause and effect: did the bacteria increase because larvae died and decayed, or did the larvae die because the bacteria increased? Walne found that in five of his six successful rearings the bacterial peak numbers were markedly lower than in his unsuccessful rearings, and suggests that the bacteria may have been influencing the successful development of his larvae, although there was no definite proof of this. He concludes that 'differences which have been observed in the value of different natural waters for rearing larvae may have their origin in the variation in the size of the bacterial flora which the water can support'. In other words the effect on the larvae of water differences is indirect via the bacteria, but it is none the less true that the waters themselves have different properties, though it may be that these only affect larvae in the confines of a dish where the bacterial population per unit volume rises higher than is possible in the open sea. Whilst this could be so it should be remembered that real changes of fauna have taken place in areas where there have been known changes of water masses and, in default of any more reasonable explanation, it would be rash to conclude that there is no real relation between the inability of water in a dish to support larvae and the larval supporting capacity of the same kind of water in the sea.

Some observations which may have a bearing on this question of the effect of excessive bacterial growths in dishes of developing larvae were mentioned in a previous paper (Wilson & Armstrong, 1954, p. 357). The antibiotics penicillin and streptomycin in various concentrations added to the water had no definite effects on the larvae, but as no bacterial counts were made it is not known whether they had any effect in reducing the bacterial flora. It may perhaps be assumed that they had, for Spencer (1952, p. 100) states that at the concentration of penicillin we used bacterial growth in peptone sea water was inhibited for many days.

Whether the bacteria are the directly responsible agents for the observed structural defects in larvae reared in unfavourable waters or not, it cannot be too strongly emphasized that the effects on the larvae are but a reflexion of real differences between the waters themselves, though of what those basic differences are we are still ignorant. In any experiment in which waters are compared, natural sea waters treated or untreated, or artificial sea waters, nothing is more striking than the uniformity of appearance in structure and movement of the larvae in all the dishes containing the same kind of water. Only on very rare occasions during the first days is there an odd dish not in conformity with the others of the same set. Larvae in another set of dishes containing a different sort of water may or may not look different from these, but if any one dish of that set is at variance so are all the rest and in the same way. Therefore we cannot but conclude that the larvae are affected structurally, and in other ways, by the water in which they develop and that we can use these larvae as test objects, though we do not as yet know what properties of the water we are testing with them, or how far we can safely apply our results to what happens naturally in the open sea.

Of the many experiments we have made we believe that the tests with mixed waters (Wilson, 1951, Expts. 5 and 8A; Wilson & Armstrong, 1952, Expt. 1) are especially significant. It will be recalled that when good and bad waters were mixed in approximately equal proportions the larvae developing in the mixtures were closely similar to those in good waters alone and much better than those in the bad waters. Any hypothesis advanced to explain our results must account for this.

SUMMARY

In 1954 and 1955 various natural, artificial and treated sea waters were tested with developing eggs of *Echinus esculentus*. The following are the main observations:

1. Sea waters filtered through Doulton candles at the time of collection and proved bacteriologically sterile until use were very little different from sea waters collected at the same time in the ordinary way and not sterile. (Expts. 1, 3, 8 and, with qualifications, 6.)

2. Undiluted Celtic sea bottom water and undiluted Celtic mud 'extract' were harmful to the eggs, but small volumes of both added to natural sea waters and to artificial sea water had a stimulating effect. (Expt. 1.)

3. In water from near the bottom at E 1 eggs did worse, in 1954, than in surface water from the same locality. In 1955 they did slightly better in the deeper water. (Expts. 3, 4 and 7.)

4. Eggs placed immediately after fertilization in water from near the bottom at E 1 (and from near the bottom at the *Echinus* ground) and transferred at first cleavage to surface waters were affected unfavourably. (Expts. 3, 4 and 7.) Artificial sea water had no such effect. (Expt. 5.)

5. The addition to natural and to artificial sea waters of filtrates from culture medium ('erdschreiber') and from thick cultures of diatoms and flagellates had no significant effects on the eggs developing in those waters. (Expt. 6.)

6. In 1955 surface water from the Clyde was a better medium for development than surface water from E 1. The difference was similar to that frequently observed several years previously. (Expt. 7.)

7. Extracts of natural sea waters made by a new process were non-poisonous, but the experiment in which they were added to artificial sea water was inconclusive owing to the eggs proving to be of poor quality. (Expt. 8.)

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SOME OBSERVATIONS ON *EVADNE NORDMANNI* LOVÉN

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(Plate I and Text-figs. 1–6)

Apstein (1910) found *Evadne nordmanni* to be the most widely distributed and in general the commonest cladoceran in the plankton of the sea areas around the coasts of Europe investigated by the International Council. It is known to occur predominantly in coastal waters and has been regarded mainly as a neritic species in the past, but considering recent records Wiborg (1955) suggests that it may also be able to establish populations in the open ocean.

Despite its importance in the marine plankton our knowledge of the biology of this species is meagre compared with that of the common freshwater Cladocera. Rammner (1930) has reviewed the earlier investigations on *E. nordmanni* and other marine species. More recent studies include those of Jorgensen (1933) and Cheng (1947), while Baker (1938) gives a detailed account of the taxonomy of marine Polyphemidae.

In view of the often inadequate material available to previous workers for the study of seasonal changes in one locality, it was decided to follow the population of *Evadne* at a station in the Clyde Sea Area during 1951 with particular attention to fluctuations in abundance, size and the mode of reproduction.

Evadne reproduces mainly by parthenogenesis, in which case the eggs are laid into the brood pouch and develop there into free-swimming young. Often some of the females carry no developing embryos but show the formation of usually one resting egg. Males are normally present in the population on these occasions and it has been generally assumed that resting eggs develop as a result of sexual reproduction. This is known to be ordinarily the case in freshwater Cladocera, with the exception of some arctic populations of *Daphnia* which produce unfertilized resting eggs.

MATERIAL AND METHODS

Quantitative plankton samples were collected at a station near the Little Cumbrae lighthouse, where the depth is between 90 and 105 m. The samples were taken with a Clarke–Bumpus plankton sampler (Clarke & Bumpus, 1940) in a series of horizontal tows, each of 10 min duration, at average depths of 1, 10, 20, 30 and 50 m. An additional tow at 40 m was made on some occasions. Unfortunately, it was not possible to tow at a constant depth, the angle of the towing wire varying between 30 and 45° to the vertical. The sampler was used fitted with a net of 200 meshes to the inch, until 7 May when it was replaced by one of 129 meshes to the inch. The volume of water filtered during a single haul varied between 2 and 4 m^3.

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Additional tow-nettings were frequently taken off Keppel Pier at Millport using larger nets of the same mesh size, and data obtained from these samples have been used when *Evadne* were scarce towards the end of the year.

All samples were preserved in 5% formalin. When necessary samples were subsampled for examination and counts using the dilution method of Russell & Colman (1931).

All measurements were made with an eyepiece micrometer on which each division represented $9{\cdot}7\,\mu$. Embryo numbers were counted after dissecting the embryos out of each parthenogenetic female.

Baker (1938) has discussed the various points of reference which have been used for the measurement of marine Cladocera and has given diagrams to illustrate what she calls the morphological length. This dimension is useful to compare the size of different species, but demands some personal judgement to determine the anterior point on the margin of the head.

In this investigation lengths have been measured from the point of attachment of the antennal elevator muscles on the crown of the head to the tip of the claws on the caudal furca (see Pl. I). This measurement approximates to the greatest length of the body excluding the brood pouch. In a very small proportion of the preserved *Evadne* there was a slight depression at the point of attachment of the antennal muscles, and measurements were adjusted to correct for this by noting the body outline on each side of the depression. The short caudal furca is capable of a slight amount of movement, but is set at a nearly constant angle to the rest of the body in preserved specimens.

VERTICAL DISTRIBUTION

The investigations made by Wiborg (1940, 1944, 1954) on the plankton of Norwegian coastal waters have shown that *Evadne* is mainly to be found living near the surface.

This was also found to be true in the Clyde. All the samples were collected during the daytime and the numbers of *Evadne* at each depth are given in Table 1. Very few occurred in hauls taken at depths greater than 30 m and, with one exception, greatest numbers were found at either the 1 or 10 m levels.

SEASONAL DISTRIBUTION

In a survey of the Clyde plankton during 1923 and 1924 Marshall (1925) records that *E. nordmanni* was common in May and June, fairly common till September and frequent in October. From a study of tow-nettings taken in the North Sea off the Northumberland coast during the years from 1921 to 1930 inclusive, Jorgensen (1933) found that the numbers of *Evadne* usually showed an early summer maximum followed by a less extensive one during September or later. She also noted the presence of males during these periods and that the numbers of resting eggs produced were roughly proportional to the numbers of individuals present.

TABLE 1. NUMBER OF *EVADNE* PER CUBIC METRE

Date 1951	1 m	10 m	20 m	30 m	40 m	50 m
9. iii.	17	9	–	–	–	–
13. iii.	6	7	0	–	–	–
29. iii.	29	27	3	0	–	0
13. iv.	36	269	30	0	–	0
19. iv.	89	85	15	1	–	0
25. iv.	91	232	31	0	–	0
7. v.	1380	64	16	0	–	0
24. v.	375	1325	269	19	–	30
29. v.	428	465	168	51	60	64
7. vi.	211	1636	672	150	–	0
14. vi.	402	359	29	0	–	–
20. vi.	422	766	177	44	–	3
29. vi.	49	764	122	26	–	1
2. vii.	511	143	25	0	–	0
11. vii.	269	278	244	45	1	2
19. vii.	209	131	10	2	0	0
24. vii.	121	126	27	0	0	0
1. viii.	91	252	67	9	0	0
7. viii.	60	138	5	0	0	0
17. viii.	111	130	350	36	4	0
3. ix.	15	4	0	0	0	0
13. ix.	0	1	0	0	0	0
17. ix.	5	0	0	0	0	0
25. ix.	5	1	0	0	0	0
11. x.	16	2	1	0	0	0
24. x.	3	2	0	0	0	0
7. xi.	0	0	0	0	0	0

– No sample taken.

Text-fig. 1 shows the average numbers of *Evadne* per cubic metre in the upper 30 m at the station in the Clyde during 1951 and also the percentages of sexual individuals in the population (i.e. males, and females showing the formation of a resting egg).

Evadne was first observed in the plankton at the end of February; quantitative sampling was begun on 9 March and continued until 7 November. The population increased reaching a maximum from May to early June and declined during the following months to a very low density in September. There was a slight recovery during October, but the species was absent from all the samples taken in November and December. The first appearance of sexual individuals coincided with the marked increase in numbers during May and sexual reproduction became most intense in October.

There was, therefore, no clear development of a second maximum and the percentage of sexual individuals showed no relationship to the population density.

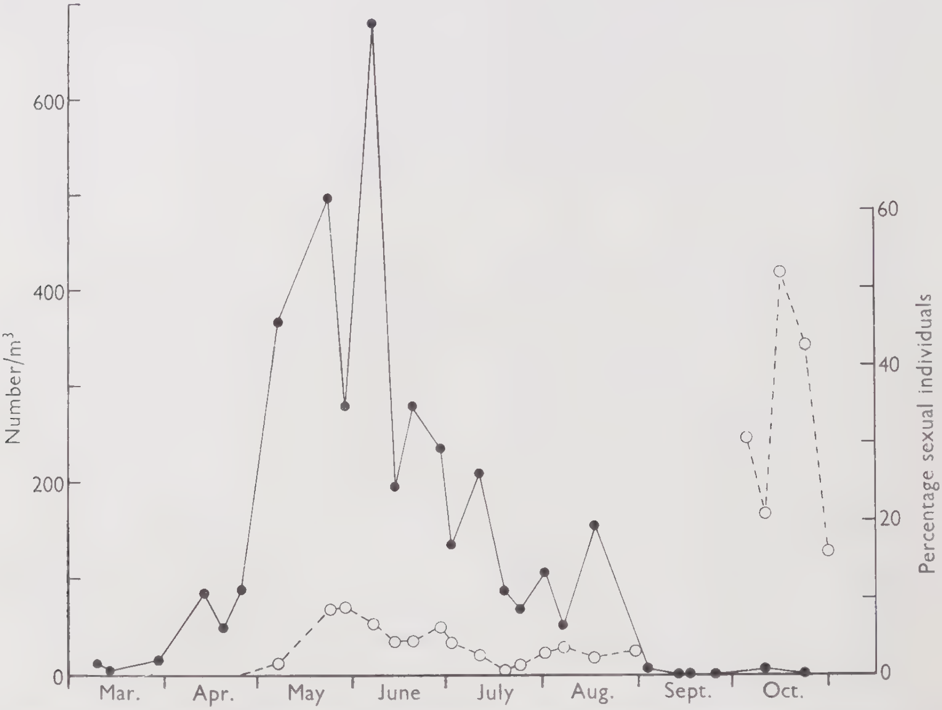

Fig. 1. Mean number of *Evadne* per cubic metre in the upper 30 m during 1951 (●——●), and the percentage of sexual individuals in the population (○– – –○).

GROWTH AND REPRODUCTION

Kuttner (1911) studied the development of the parthenogenetic eggs of *E. nordmanni* and found that when embryos reach an advanced stage of development and show the first traces of eye pigmentation, their own eggs mature and pass into the embryonic brood space. By the time the young are liberated from the brood pouch of the mother these eggs have segmented to a blastula stage with a large cleavage cavity. The young are in effect miniature adults.

Attempts were made to rear individual animals in order to determine the usual number of instars between birth and the liberation of the first brood of young, but proved unsuccessful. Two of the major problems are that *Evadne* tends to stick to any water-air surface film and since it is not a filter-feeder conditions suitable for feeding are difficult to produce.

In order to provide some information on the relation between size and reproductive state two large samples of *Evadne* were examined. The length of each individual was measured and the stage of embryonic development in the parthenogenetic females noted. Two easily recognizable stages have been selected and are arbitrarily classed as early and late stages. They are defined as follows:

Early. Embryos roughly spherical, though sometimes distorted owing to being pressed together in the brood pouch. They show no signs of elongation or the appearance of the second antennal rudiment.

Late. Embryos at an advanced stage of development and showing at least the first traces of eye pigmentation. All the limbs are present and the dorsal pouch is developing so that the embryo lies on its side when removed from the brood pouch.

The results obtained from the two samples are shown as percentage size-frequency distributions in Text-fig. 2 and the stages referred to are illustrated in Pl. I.

Text-fig. 2A shows the size distribution of all parthenogenetically reproducing females and Text-fig. 2B the size distribution of those with embryos at the stages of development described above. Primiparae carrying early embryos can be clearly distinguished from older females with a second or subsequent brood of early embryos on the basis of size, shape of the carapace and position of the embryos within the dorsal pouch formed by the carapace (Pl. I, figs. 1, 4). In view of the considerable overlap in sizes it proved impossible to separate females with late-stage embryos (Pl. I, fig. 2) into primiparae and older individuals. The striking change in the outline of the brood pouch of females following the emergence of young is illustrated in Pl. I, figs. 3, 4.

Text-fig. 2C shows the size distribution of males (Pl. I, fig. 5) and females producing a resting egg (Pl. I, fig. 6), presumably after fertilization. When present, these sexual females are among the largest individuals in a sample; they fall within a higher size range than the parthenogenetic females with late-stage embryos, and more closely correspond in size distribution to females with second or subsequent broods of early-stage embryos. This is shown only in the sample collected on 29 May since, when sexual reproduction is intense as on 5 October, older females with early embryos are very scarce. At their birth young females are carrying developing embryos and it would appear that at least one brood of young is first liberated before a resting egg can be produced.

Rammner (1930) noted the large size of the sexual females, but since they differ from the parthenogenetic females in the nature of the brood-pouch epithelium and have a carapace opening, he was of the opinion that they must be large at birth and do not first produce a parthenogenetic brood.

Because of the size range of sexual females and the fact that all embryos nearing the stage of liberation can be distinguished either as males or parthenogenetic females with blastulae, Rammner's suggestion is untenable. A new

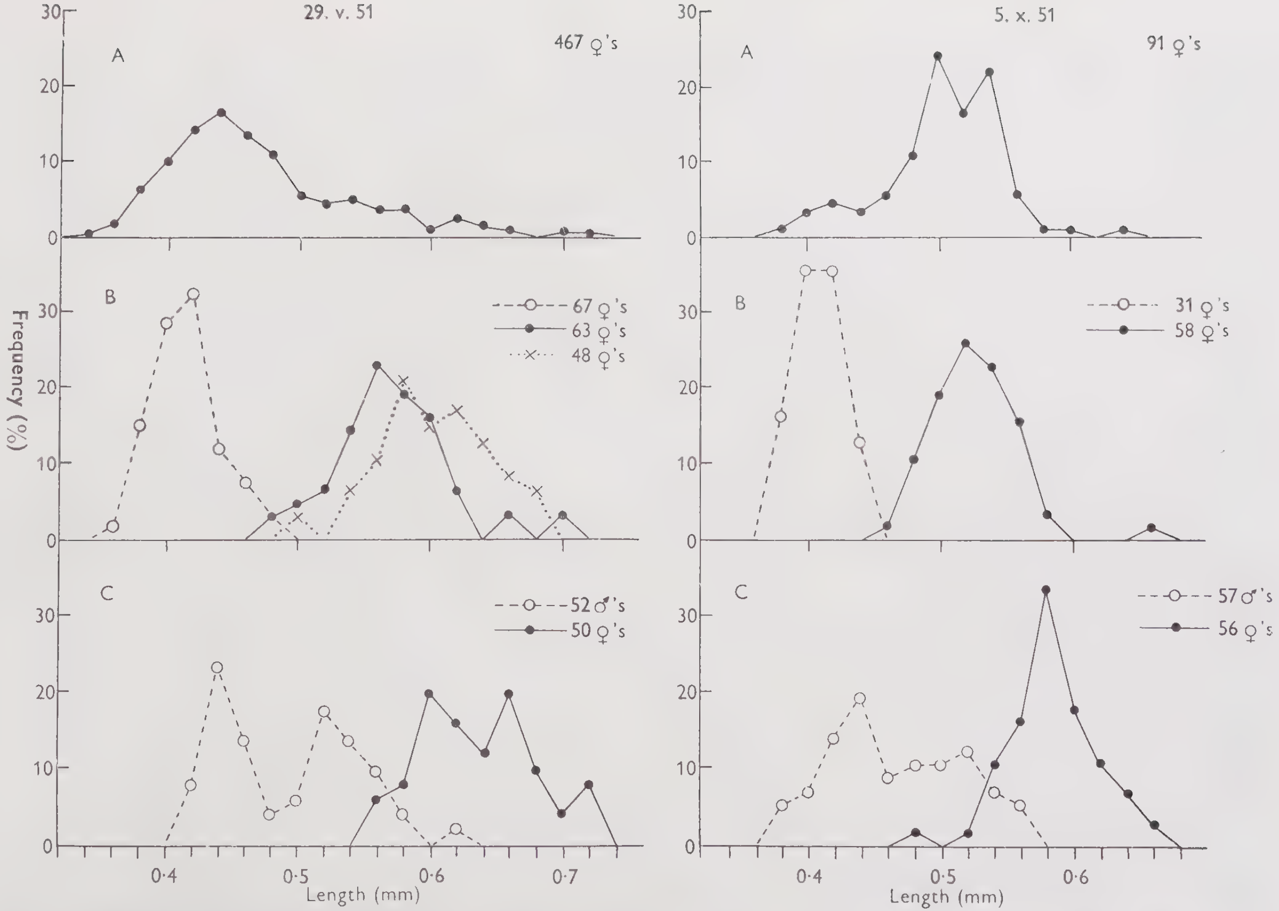

Fig. 2. Percentage size-frequency distribution of *Evadne*. A: parthenogenetic females. B: primiparae with early-stage embryos (○- - -○), females with late-stage embryos (●——●), and females with a second or later brood of early-stage embryos (× · · · · ×). C: males (○- - -○), and females showing the formation of a resting egg (●——●).

carapace is formed at the liberation of a brood of young as shown in Pl. I, fig. 3, and the carapace opening might possibly appear at this time. The shape of the carapace of females showing the first signs of resting-egg formation is also very similar to that of females with second or following broods of early embryos.

The fate of mature resting eggs can only be surmised. Jorgensen (1933) noted cast exoskeletons containing resting eggs in the plankton on several occasions, but these were never observed in the Clyde plankton. Nor, in fact, were any females with mature resting eggs ever found showing signs of an incipient moult. It therefore seems possible that the majority sink to the bottom after the death of the parent.

EMBRYO NUMBER AND SIZE

Cheng (1947) found that larger female *Evadne* generally have more embryos than smaller females. His results cannot, however, be accepted as conclusive, since he groups together individuals from several samples collected over a considerable period. Increased egg production with an increase in body size has been demonstrated in several species of *Daphnia* and other freshwater Cladocera by Green (1954, 1956).

In Text-fig. 3 the number of embryos is plotted against maternal length in two large samples. The means, standard deviations and ranges for both length and embryo number of females with embryos at the two stages defined are also shown.

The mean embryo numbers in the sample collected on 29 May are roughly constant in the lower size-groups, which represent only primiparae and increase in the higher size-groups, since these include older females with early-stage embryos.

In the sample taken on 9 March the embryo number shows a decrease with increase of size, individuals with late-stage embryos having fewer than the primiparae with early-stage embryos. This is at least partly due to a proportion of the embryos present at birth failing to develop, since disintegrating embryos and undifferentiated patches of tissue could be clearly seen in the brood pouches of some females. In preserved specimens these abnormal embryos are darker in colour, very fragile and occasionally recognizable as being at an earlier stage of development than normal embryos in the same brood pouch. As in other Polyphemidae the eggs of *Evadne* contain very little yolk, development depending on nourishment supplied by the mother through the fluid bathing the embryos in the closed brood pouch (Kuttner, 1911; Rammner, 1930). The loss of embryos could therefore be due to the effect of adverse conditions on the parents.

The apparent disintegration and resorption of embryos was observed in the samples collected 9 March, 13 April, 14 June and 30 October. Text-fig. 5B shows that in many of the samples collected during the year the brood number of females with late-stage embryos was smaller than that of the primiparae with early embryos, despite the fact that the former group will include a small and unknown proportion of individuals producing a second or even later brood.

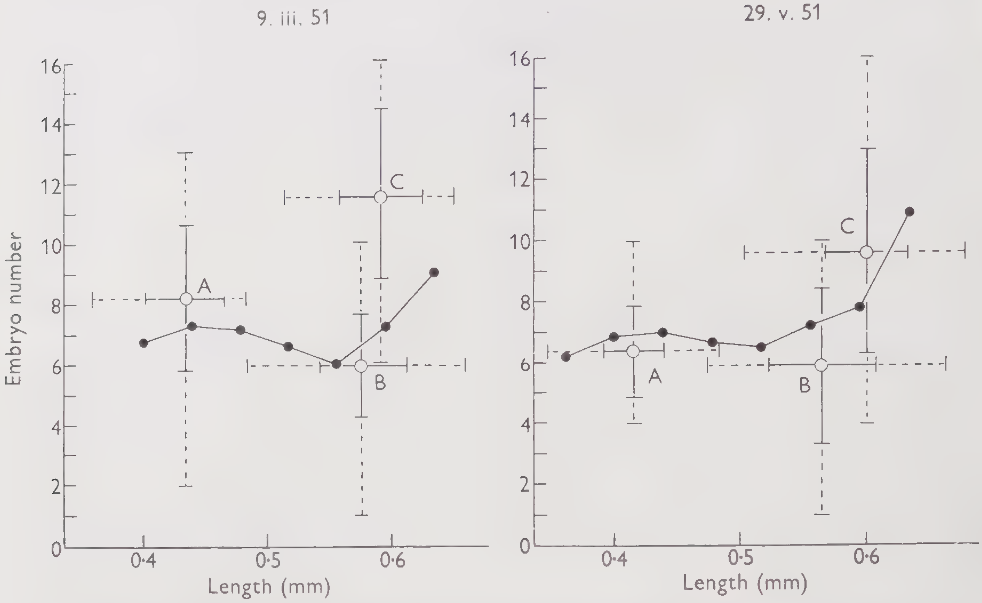

Fig. 3. Embryo number and size. Also shown are the means, standard deviations and ranges for embryo number and size of primiparae with early-stage embryos (A); females with late-stage embryos (B); females with a second or later brood of early-stage embryos (C).

Females with second and subsequent broods of early-stage embryos were very scarce in some of the samples and insufficient were obtained for the embryo numbers to be plotted on a seasonal basis. Their embryo numbers do, however, tend to fluctuate with those of females with late-stage embryos (Text-fig. 4).

It is clear that the best measure of the average production of young per brood can be obtained when only those individuals with late-stage embryos are considered.

Although the number of late-stage embryos is very variable and the range of sizes is rather small, larger individuals tend to produce larger broods of young. This is shown in Table 2 for several samples selected to cover a wide range of reproductive capacities.

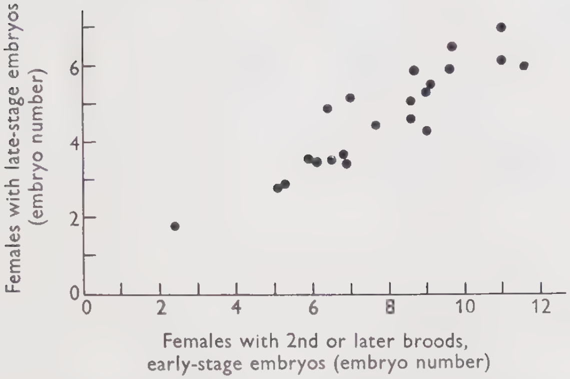

Fig. 4. Scatter diagram showing the relation of the mean embryo number of females with late-stage embryos and the mean embryo number of females with a second or later brood of early-stage embryos for each sample.

TABLE 2. SIZE AND EMBRYO NUMBER OF FEMALES WITH LATE-STAGE EMBRYOS

Date 1951	Length (mm.)*	No. examined	No. of embryos Max.	Min.	Mean
9. iii.	0·49	4	6	2	4·0
	0·54	25	8	1	5·2
	0·59	36	10	3	6·4
	0·64	9	9	5	7·2
13. iv.	0·54	4	4	2	3·0
	0·59	20	6	2	3·4
	0·64	18	6	2	3·5
	0·69	6	5	1	3·7
	0·74	2	5	1	3·0
29. v.	0·49	8	8	2	5·1
	0·54	24	9	1	5·2
	0·59	27	10	1	6·5
	0·64	2	10	5	7·5
	0·69	2	10	7	8·5
7. vi.	0·49	11	5	1	2·1
	0·54	28	8	1	3·2
	0·59	5	6	2	3·8
	0·64	1	—	—	5·0
5. x.	0·44	1	—	—	2·0
	0·49	22	7	1	3·7
	0·54	31	7	3	5·1
	0·59	3	8	6	7·0
	0·64	1	—	—	5·0
31. x.	0·49	14	4	1	2·7
	0·54	18	5	1	3·2
	0·59	6	6	1	4·1
	0·64	2	6	5	5·5

* Class mid-point.

FLUCTUATIONS IN LENGTH AND EMBRYO PRODUCTION DURING THE YEAR

Green (1956, q.v. for further references) has summarized the main results of experimental work carried out to study the effect of environmental factors influencing growth and egg production of several species of the freshwater genus *Daphnia*. Of these factors, temperature and food supply are likely to be the most important which affect Cladocera living near the surface of the sea. In general terms, experimental work on cultures of *Daphnia* has shown two features: (i) At low temperatures individuals increase in size more slowly, but reach a larger final size than those living at higher temperatures. A certain range of temperature is favourable to egg production, above and below which there is a considerable diminution of the number of eggs produced. (ii) Starvation both decreases growth and reduces egg production. Green (1956) gives evidence that it also reduces egg size, which in cladocerans that do not secrete a nutrient fluid is directly related to the size of young at birth.

The samples of *Evadne* from the Clyde Sea Area during 1951 show that there were considerable fluctuations in size and the production of young. Text-fig. 5 shows the changes in the mean length and embryo number of females with late-stage embryos and primiparae with early-stage embryos. The mean lengths of these two classes of females show similar fluctuations and there is a notable fall from the end of May to late July. During this same period the mean number of late-stage embryos showed initially a sharp decline followed by a more steady rise. In early August, shortly after the recovery of embryo production, there was an increase in mean lengths, though they never attained their former level.

The seasonal variation in length of the two classes of females indicates that the size at which females had embryos nearing the stage of liberation was mainly dependent on their own size at birth, and that there were not usually any pronounced changes in the growth increment. A possible explanation of the relationship between fluctuations in the length of females and numbers of late-stage embryos produced, is that both are to a certain extent influenced by the same environmental conditions, but that changes in the size of young at birth are more gradual and lag behind fluctuations in the number of young produced per brood.

The fluctuations described can be correlated with surface sea temperatures at Keppel Pier, Millport, shown in Text-fig. 5c. In March, April and May females were larger than at any other time during the year. This may possibly be related to the lower sea temperatures during the spring, though otherwise there are no clear connexions since a partial recovery of size occurred during August when the temperature was about at a maximum for the year.

Both length and embryo number were better correlated with the amount of microplankton caught by the net, suggesting that food supply may be the more important factor. The greatest quantities of microplankton occurred from 13 April to 24 May and again from 24 July to 17 October. Details of the relationship to food supply will be discussed in a following section.

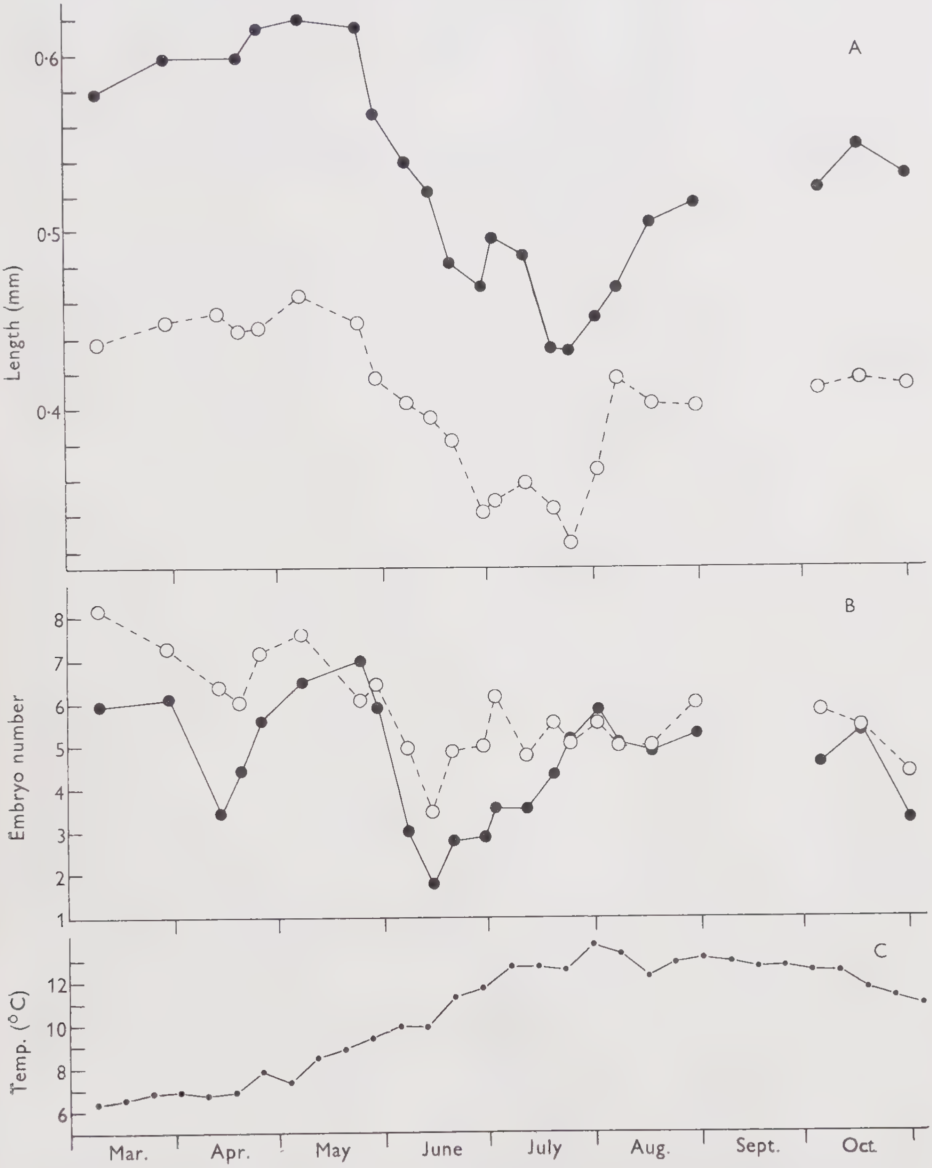

Fig. 5. Fluctuations in mean length (A), and mean embryo number (B), of primiparae with early-stage embryos (○- - -○), and females with late-stage embryos (●——●) (C). Surface sea temperatures at Keppel Pier, Millport, as 8-day means.

PARTHENOGENETIC AND SEXUAL REPRODUCTION

Berg (1931) carried out a detailed study of changes in the mode of reproduction of several species of *Daphnia* both in natural populations and cultures. The results of this work clearly support his hypothesis that the transition from parthenogenesis to gamogenesis is caused by the influence of unfavourable external conditions, the environmental factors producing a state of depression in the females and thereby changing the mode of reproduction and the sex of the young. One of the most obvious signs of depression shown by the parthenogenetic females is a reduction in the mean number of eggs produced and this takes place before each sexual period.

Observations on *Evadne* made by Jorgensen (1933) have been discussed by Berg (1936) who finds a close agreement with his own on freshwater daphnids. Jorgensen notes that developing resting eggs first made their appearance in the samples when the population consisted to a great extent of less bulky more triangular forms producing a smaller number of embryos than usual. Cheng (1947) examined five samples of *Evadne* collected during two summers and found an approximate inverse relationship between the mean embryo number and the percentage of sexual individuals in the population.

It has been shown that the mean number of embryos per female found in a sample of the total population will depend to a great extent on the proportions of the various stages of development, and that a better measure of the reproductive capacity of the parthenogenetic females can be obtained if only those with late-stage embryos are considered. The results obtained in the Clyde are shown in Text-fig. 6 and Table 3, in which fluctuations in the mean number of embryos carried by these females can be compared with the percentage of males in the population and the percentage of females showing the formation of a resting egg.

Males first appeared in the samples when the mean number of embryos was relatively high and the percentage of males decreased during the drastic fall of mean embryo number in June. By far the greatest percentage of males occurred during October when the mean embryo number was higher than in June and early July. At the end of October when the percentage of males decreased this was accompanied by a decrease in the mean embryo number.

Since *Evadne* were very scarce during September no adequate data are available on the mean number of late-stage embryos, but the embryo numbers of all females found in the samples suggest that there was no pronounced decrease in the reproductive capacity during this month (Table 3).

The sex of many of the late-stage embryos can be determined and sufficient numbers were available in the samples collected 5 October and 31 October to show that male production was greater on the former date, confirming the evidence of the different percentage of males in the two populations (Table 4).

These results do not admit a simple explanation on the basis of Berg's depression hypothesis. It is possible, however, that the first appearance of males in the samples is related to a period of depression indicated by the lower mean embryo numbers found in previous samples during April. Later fluctuations in the percentage of males might also be accounted for if it was considered that moderate depression favours greater male production than severe depression. To explain the findings of Stuart, Cooper & Coady (1933) that under conditions of severe depression female *Moina* become what they term 'sex fast' and produce female young only, Berg (1936) suggests that severe depression might have a different effect from moderate depression. It is not, however, known whether sex-fast females occur in natural populations of freshwater Cladocera.

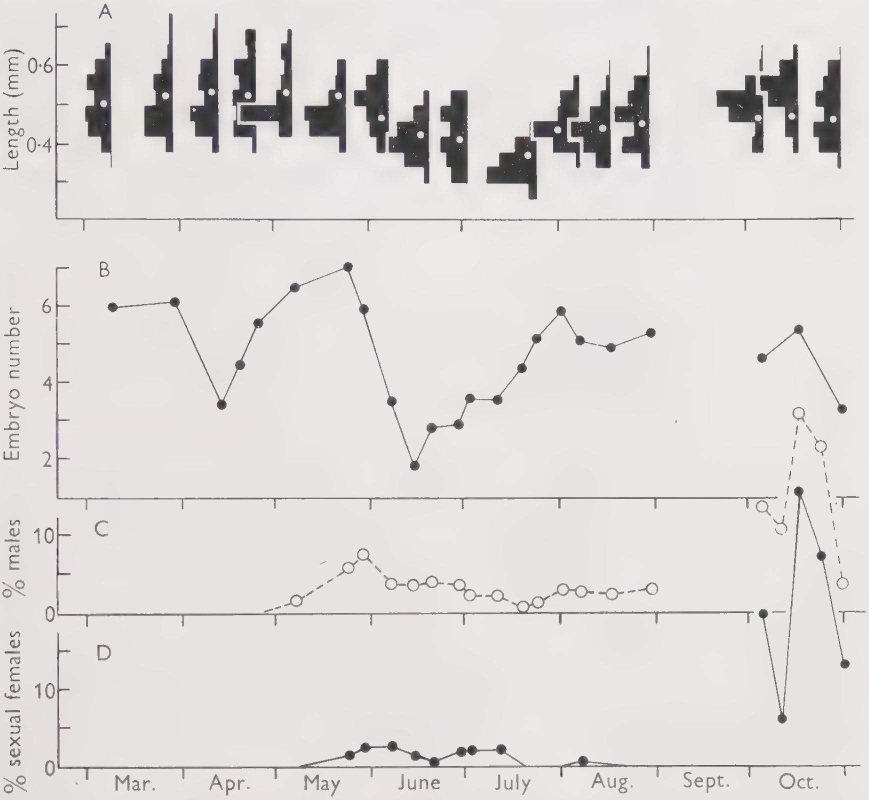

Fig. 6. A: percentage size-frequency distribution of females on some of the dates of sampling. The open circles within the histograms indicate the intermediate size between the means for primiparae with early-stage embryos and females with late-stage embryos. B: fluctuations in the mean number of late-stage embryos. C: percentage of males in the population. D: percentage of females showing the formation of a resting egg (sexual females).

Fluctuations in the percentage of females showing the formation of a resting egg follow those of the males, but will give a distorted picture of

TABLE 3. LENGTH, EMBRYO NUMBER AND PERCENTAGE OF SEXUAL INDIVIDUALS

(Figures in parentheses give number of individuals used in calculating the mean or percentage.)

Date 1951	Parthenogenetic females. Mean embryo no.	Primiparae with early-stage embryos		Females with late-stage embryos		Females with a second or later brood of early-stage embryos. Mean embryo no.	Sexual individuals	
		Mean length (μ) and S.D.	Mean embryo no. and S.D.	Mean length (μ) and S.D.	Mean embryo no. and S.D.		% males in population	% females with a resting egg
9. iii	7·1 (344)	436 ± 31	8·2 ± 2·4 (102)	578 ± 35	6·0 ± 1·7 (74)	11·6 (38)	0	0 (800)
29. iii	7·6 (97)	449 ± 22	7·3 ± 1·5 (36)	598 ± 41	6·1 ± 2·0 (16)	11·0 (4)	0	0 (510)
13. iv	5·5 (95)	454 ± 29	6·3 ± 0·9 (32)	622 ± 45	3·4 ± 1·2 (50)	6·9 (10)	0	0 (303)
19. iv	5·7 (97)	443 ± 26	6·0 ± 1·4 (18)	598 ± 18	4·4 ± 1·4 (16)	7·7 (4)	0	0 (202)
25. iv	6·7 (133)	445 ± 28	7·1 ± 1·3 (33)	616 ± 30	5·5 ± 0·9 (17)	9·1 (6)	0	0 (344)
7. v	7·0 (77)	464 ± 22	7·6 ± 1·1 (28)	620 ± 27	6·5 ± 0·8 (11)	9·7 (4)	1·5	0 (198)
24. v	6·4 (119)	448 ± 23	6·1 ± 0·8 (38)	616 ± 36	7·0 ± 1·2 (22)	11·0 (2)	5·7	1·4 (846)
29. v	7·0 (467)	417 ± 24	6·4 ± 1·5 (67)	566 ± 42	5·9 ± 2·6 (63)	9·6 (48)	7·4	2·3 (876)
7. vi	4·4 (130)	404 ± 36	4·9 ± 1·2 (16)	539 ± 34	3·0 ± 1·7 (45)	6·1 (9)	3·7	2·5 (539)
14. vi	2·7 (99)	395 ± 17	3·5 ± 0·9 (30)	525 ± 22	1·8 ± 1·0 (23)	2·4 (14)	3·5	1·4 (736)
20. vi	4·6 (127)	382 ± 32	4·9 ± 1·3 (35)	482 ± 34	2·8 ± 1·3 (27)	5·1 (10)	3·9	0·4 (908)
29. vi	5·4 (90)	341 ± 27	5·0 ± 1·1 (20)	469 ± 48	2·9 ± 1·1 (28)	5·3 (3)	3·5	1·9 (693)
2. vii	5·7 (107)	348 ± 33	6·1 ± 0·7 (23)	497 ± 34	3·5 ± 1·4 (20)	6·5 (12)	2·2	1·9 (495)
11. vii	4·6 (113)	359 ± 38	4·8 ± 1·2 (33)	487 ± 40	3·5 ± 1·8 (31)	5·9 (14)	2·1	0·7 (418)
19. vii	5·7 (99)	344 ± 37	5·5 ± 0·8 (28)	434 ± 29	4·4 ± 0·8 (21)	9·0 (5)	0·7	0 (137)
24. vii	5·2 (91)	324 ± 22	5·1 ± 0·8 (31)	433 ± 26	5·2 ± 0·9 (21)	7·0 (2)	1·2	0 (264)
1. viii	5·7 (106)	366 ± 37	5·6 ± 0·7 (23)	452 ± 30	5·9 ± 0·5 (16)	8·7 (3)	2·9	0 (204)
7. viii	5·5 (103)	417 ± 43	5·0 ± 1·1 (19)	468 ± 45	5·1 ± 0·9 (33)	8·6 (13)	2·6	0·5 (188)
17. viii	4·9 (107)	403 ± 32	5·0 ± 0·8 (43)	506 ± 55	4·9 ± 1·0 (19)	6·4 (8)	2·2	0 (368)
30. viii	6·1 (126)	401 ± 24	6·0 ± 0·5 (18)	518 ± 19	5·3 ± 1·0 (7)	9·0 (8)	2·9	0 (177)
3. ix	6·0 (45)	—	—	—	—	—	0	0 (45)
13. ix	5·0 (2)	—	—	—	—	—	0	0 (2)
17. ix	5·8 (8)	—	—	—	—	—	0	0 (8)
25. ix	5·7 (12)	—	—	—	—	—	0	0 (15)
5. x	5·4 (103)	411 ± 16	5·9 ± 0·7 (31)	525 ± 31	4·6 ± 1·1 (58)	8·6 (7)	13·7	19·6 (490)
11. x	7·3 (28)	—	—	—	—	—	10·7	6·0 (56)
17. x	5·8 (102)	418 ± 13	5·5 ± 0·8 (15)	550 ± 27	5·4 ± 1·8 (27)	9·0 (3)	25·9	35·6 (216)
24. x	5·8 (17)	—	—	—	—	—	21·4	27·2 (28)
31. x	4·3 (107)	414 ± 20	4·4 ± 0·9 (31)	532 ± 36	3·3 ± 1·7 (40)	6·8 (11)	3·5	12·9 (136)

changes in the intensity of sexual reproduction, since from the size distribution of whole populations it is clear that there is a considerable variation in the number of primiparae present on the different dates of sampling and these do not produce resting eggs.

Changes in the percentage size distribution of the parthenogenetic females during the year are illustrated in Fig. 6A. From March to August major increases and decreases in the mean number of late-stage embryos tend to be followed by similar changes in the proportions of small individuals in the population. The preponderance of the larger size groups of females during October may be partly accounted for by the high production of males and resting eggs, since this will reduce the number of young females entering the population.

TABLE 4. THE SEX OF VERY ADVANCED EMBRYOS

(A single brood may contain both male and female embryos.)

Date 1951	No. of females with advanced embryos examined	No. of females with male embryos	Total no. of embryos	% of male embryos
5. x.	50	16	229	27·4
31. x.	33	4	111	9·9

The results described are at variance with the observations of Jorgensen (1933). The samples used by Jorgensen appear to have been taken with nets which would tend to select the larger size groups. She has noted that during the two periods of maximum abundance usually found during a year, the great increase in numbers was produced, as far as the samples were concerned, by a temporary influx of adults and states that no doubt immense numbers of young forms were present though not taken by the nets. It is possible, therefore, that populations consisting entirely of small individuals, similar to those found in the Clyde during June and July 1951, would not be adequately represented in the samples she examined.

The data obtained by Cheng (1947) is insufficient to be accepted as conclusive (see page 360). In Table 3 the mean embryo number of all parthenogenetic females can be compared with the percentage of sexual individuals in the population for each date of sampling in the Clyde during 1951. The values show no inverse relationship and therefore do not support Cheng's results.

FOOD AND FEEDING HABITS

Rammner (1930) states that *Evadne* is a predator, feeding on small organisms which are captured by means of its powerful raptorial legs, but notes that no direct observation had been made of the taking up of food by the living animal. He suggests that they may also feed on organic detritus. Previously Lebour (1922) had recorded the cells and spores of *Phaeocystis* from the guts of two *Evadne*.

The gut contents of both living and preserved *Evadne* were examined on several occasions, and though soft reddish or brownish debris was often present at the posterior end of the gut, no recognizable remains were found of any organisms eaten.

Some of the individuals examined were, however, found to be holding a variety of small organisms, either grasped between the endites of the second and third legs or pressed between the labrum and the mouth. Of all those measured and dissected from the plankton-sampler catches during the year, 2·1 % were holding tintinnids, 1·4 % *Peridinium* and *Goniaulax* species and 0·1 % small copepod eggs. Tintinnids were most frequently present during the period from March to May and again during August and September, peridinians during June and July. Considerable quantities of *Skeletonema* and *Thalassiosira* were present in the plankton samples during the spring, but these diatoms were not found between the legs or mouth parts of the *Evadne*. During the investigation it became evident that the proportion of individuals holding microplankton organisms depended to a great extent on the treatment of the catch after hauling the net. On the whole, greater proportions were found in the non-quantitative samples which had been fixed immediately than in the quantitative samples which were not fixed for several minutes due to time spent in washing down the net. Living *Evadne* examined only a short time after collection were rarely observed holding any organisms.

A more detailed study of the feeding habits was made on two occasions towards the end of the year. *Evadne* were obtained from 10 min hauls with 50 cm medium-mesh tow-nets (48 meshes to the inch) taken at 4 h intervals through 24 h. Two nets were fished 25 fathoms apart on a trawl warp, to the end of which was attached a cable depressor. Rigged in this way, the upper net fished at 1–2 m and the lower at 33–38 m. These hauls were taken at a station just outside Tarbert, Loch Fyne. The catches were fixed in formalin as quickly as possible after hauling the nets. During the last series water samples were collected from a depth of 1 m and the number of organisms in the samples estimated by a method previously adopted by Marshall (1947).

On both occasions very few *Evadne* were caught by the lower net and there was no evidence of vertical movements to or from the surface throughout the 24 h. Results of observations are shown in Table 5, in which the number of *Evadne* per haul with the upper net and the percentage of these holding small organisms is given. From the marked difference between the percentages in the day and night hauls it is clear that small organisms were being captured almost exclusively during the hours of daylight. Since *Evadne* possesses a relatively large compound eye, this suggests that food organisms are normally taken by an act of capture depending on sight.

Table 6 shows the percentage composition of these organisms found held by *Evadne* and the percentage composition of the microplankton in the sea at 1 m on 17–18 October. In the series of samples taken on 15–16 August as many as five tintinnids and peridinians were sometimes held by a single individual, but no *Ceratium longipes* (Bailey) were so found, although large numbers were caught by the nets. *C. furca* (Ehrenberg) was the most commonly held prey on 17–18 October, but not the most abundant dinoflagellate in the sea as estimated from the water samples. Peridinian plates and fragments of tintinnid cases were sometimes found near the mouth but never in the gut. The lack of any skeletons or hard parts of food organisms in the guts seems to indicate that only the cell contents are ingested and the hard remains later discarded.

TABLE 5. PERCENTAGE OF PRESERVED *EVADNE* FOUND GRASPING SMALL ORGANISMS IN SAMPLES COLLECTED AT 4-HOUR INTERVALS

(Figures in parentheses give the numbers examined which in each case was the total number found in the sample.)

Time (h) ...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
15 and 16. viii. 51	34·7 (121)	51·6 (250)	7·1 (294)	2·7 (150)	0 (152)	38·1 (134)	50·5 (198)
17 and 18. x. 51	19·6 (61)	25·9 (77)	0 (60)	0 (89)	0 (106)	27·3 (66)	20·4 (132)
			Hauls taken in darkness				

TABLE 6. THE PERCENTAGE COMPOSITION OF ORGANISMS HELD BY PRESERVED *EVADNE*

(The percentages for 17 and 18. x. 51 are compared with the percentage composition of the microplankton at 1 m.)

	Organisms held by *Evadne*		Microplankton
	15 and 16. viii. 51	17 and 18. x. 51	17 and 18. x. 51
Phytoplankton			
Diatoms	0·2	—	0·03
Peridinium spp.	18·2	20·9	3·0
Peridinium spp. fragments	2·7	1·6	—
Ceratium furca	0·2	56·4	34·6
C. furca fragments	—	15·0	—
Ceratium spp. less *C. furca*	—	—	55·1
Other dinoflagellates	—	1·6	6·6
Zooplankton			
Tintinnopsis spp.	73·2	1·6	0·06
Tintinnopsis spp. fragments	1·3	—	—
Small copepod eggs	1·3	3·2	0·5
Copepod egg membranes	0·7	—	—
Copepod nauplii	0·2	—	—
Larval lamellibranchs	0·9	—	—
Unidentified debris	0·9	—	—
Number of microplankton organisms examined	444	62	—
Mean number of organisms per litre	—	—	12,760

The percentages of the various groups held by a sample of *Evadne* may not reflect the relative quantities of those eaten. Armoured organisms may be eaten more slowly than fragile or naked forms, while certain types may be rejected after capture. There is also the possibility that more active prey if undamaged may escape during the fixation of the catch. However, the results suggest that *Evadne* is a selective feeder, shape and size being two of the factors determining suitable food organisms. The majority are fairly compact bodies with average dimensions of between 30 and 120 μ. *Ceratium furca* is an exception, but it differs from the other ceratia of the Clyde plankton in that the two posterior spines are rather short and point straight backwards—a factor possibly influencing its selection by *Evadne*. It is also noteworthy that most of the food organisms are motile forms.

DISCUSSION

Information obtained on the food and feeding habits of *Evadne* helps towards an interpretation of several other aspects of the biology of the species.

During the two 24 h series of tow-nettings feeding was limited to the hours of daylight which indicates that light may be necessary for the capture of food organisms. This would also account for the species being an epiplanktonic form and mainly living in the illuminated surface layers of the sea. Another implication is that in temperate regions during the winter, when most groups of microplankton are poorly represented, the time available for feeding and the depth at which prey can be seen and captured will also be limited. When these adverse conditions are considered, the ability to produce resting eggs appears to be a great advantage in ensuring that the population is carried through to the following spring.

Observations indicate that *Evadne* is a selective feeder and that peridinians and tintinnids are the most important food organisms. The results of several comprehensive plankton investigations show that fluctuations in the population density of *Evadne* can often be correlated with variations in the abundance of *Peridinium* spp. and dinoflagellates of a shape and size similar to those eaten by *Evadne* in the Clyde Sea Area.

The tables given by Dakin & Colefax (1933) to illustrate the cycle of plankton in the coastal waters of New South Wales during 1931, indicate that the periods of maximum abundance of each of the three species of *Evadne* recorded were also the periods when highest concentrations of *Peridinium* spp. and *Ceratium furca* occurred. Greatest numbers of *Evadne nordmanni* were recorded on the same date as the maxima for these dinoflagellates. Accounts of the zooplankton (Deevey, 1956) and phytoplankton (Conover, 1956) of Long Island Sound during 1952, show that *E. nordmanni* was only recorded within the period when *Peridinium* spp. were present in other than trace quantities, and that it occurred in greatest numbers towards the end of a flowering of these species. Lohmann (1908) gives complete tables for the plankton in Kiel Bay during 1905–6. The two species of *Evadne* recorded were always very scarce, nevertheless, each small peak in numbers followed within 3 weeks of a maximum of *Peridinium* spp. excluding *P. triqueta* (Stein). This latter species was the dominant peridinian in Kiel Bay, but according to Lohmann's estimate of the average cell volume, smaller than any organism noted as captured by *Evadne* in this present investigation.

Although in all the reports of comprehensive plankton investigations examined significant increases in the population of *Evadne* could be correlated with flowerings of *Peridinium* spp., it is clear that factors other than food supply at times limit the population density. This is illustrated by the seasonal cycle of plankton at the Borkumriff Lightship station during 1910, described by Lücke (1912). Here the increase in numbers of *Evadne nordmanni* during April and May closely followed a flowering of *Peridinium* spp. After May numbers of *Evadne* declined and the species finally disappeared from the plankton in August, despite the fact that *Peridinium* spp. showed a second increase during July and remained fairly abundant until the end of October. A rather similar sequence of events occurred in the Clyde plankton during 1923, described by Marshall (1925). *Evadne nordmanni* was common during May and June and *Peridinium* spp. showed a maximum in May after the April diatom flowering. Fewer *Evadne* were recorded after June and there was no increase during or immediately after the second peak of *Peridinium* spp. in August.

All the investigations referred to reveal no clear relationship between the fluctuations in the numbers of *Evadne* and those of the Tintinnoinea when data on this group are available. Several recent studies, for example, that made by Digby (1953) on plankton production in Scoresby Sound, have, however, shown that tintinnids are often most abundant at about the same time as the peridinians, both groups being associated with the late phase of a phytoplankton outburst.

Only qualitative observations were made on the net-caught microplankton in the Clyde Sea Area during 1951. It is, however, possible to state that both peridinians and tintinnids were present in greatest quantities from early April to the end of May and from late July to mid-October. The first period of abundance coincided with the spring diatom flowering and the second with the period when ceratia were predominant. Fluctuations in both length and the mean number of late-stage embryos of *Evadne*, as shown in Fig. 5 (p. 359), may therefore reflect changes in the density of food organisms. Variations in embryo production might be expected to affect in turn the population density of the species as shown in Fig. 1 (p. 352). The rapid growth of the population during May took place when embryo production was highest and numbers decreased shortly after embryo production had declined. However, the population density was very low in September, although embryo production had recovered the previous month. Such a situation might be accounted for if during the summer and autumn the population of *Evadne* was being greatly reduced by predation. This appears to be a reasonable hypothesis, since at

Borkumriff during 1910 (Lücke, 1912) and in the Clyde during 1923 (Marshall, 1925), two cases when the population of *Evadne* did not respond to an increase of food supply during the summer, known zooplankton feeders: coelenterates, chaetognaths and larger crustacean larvae, were more abundant at this time than during the spring. In this connexion it may be relevant that *Evadne* is a slow and rather weak swimmer compared with the copepods, and consequently may perhaps be more easily caught by the larger predatory animals.

In the Clyde during 1951 there was no such clear relationship between the number of young produced per brood by the parthenogenetic females, and the occurrence of gamogenesis as that described in natural populations of *Daphnia* by Berg (1931) and Green (1955). It has been suggested that in the case of *Evadne*, moderate rather than severe depression, to judge by size and production of young, promotes gamogenesis. Comparing the development of the parthenogenetic and resting eggs in *Daphnia* and *Evadne* it does not seem unlikely that gamogenesis will be influenced by different degrees of depression in each case. The resting eggs of *Evadne* are large in relation to the size of the female producing them, and also *Evadne* does not have the capacity for fat storage in the ovary and surrounding tissues possessed by *Daphnia* species. Most of the nourishment necessary for the formation of the large and heavily yolked resting egg will, therefore, depend on the immediate food supply available to the parent during the period it is being produced. In view of the lack of food reserves it would seem advantageous to the species if resting egg formation took place before any drastic decrease in the level of nutrition, causing severe depression and possibly leading to the extinction of the population.

I wish to thank the Director and Staff of the Marine Station, Millport, for providing every facility required and for much assistance. I am especially grateful to Dr S. M. Marshall and Dr A. P. Orr for the encouragement and helpful advice they have given me during the course of this work. The research was carried out during the tenure of a Colonial Fisheries Research Studentship.

SUMMARY

During 1951 *Evadne nordmanni* was present in the Clyde plankton from late February to the end of October and was most abundant during May and early June. The bulk of the population was always found in the top 30 m. Reproduction is mainly parthenogenetic; sexual individuals first appeared in May and sexual reproduction was most intense during October.

The relation between maternal size and stage of embryonic development is described. Sexual females showing the formation of a resting egg are large and have previously produced at least one brood by parthenogenesis.

Of the females with embryos at an early stage of development the primiparae have smaller broods than larger and older individuals. During the development of embryos some may be resorbed and in the primiparae this results in a decrease of embryo number with increase of size. Larger individuals tend to produce larger broods of young.

There was a considerable decrease of size from June to July and a partial recovery in August. These fluctuations follow similar fluctuations in embryo production.

The reproductive capacity of the parthenogenetic females and the intensity of sexual reproduction did not show any clear relationship, though there are indications that the latter is favoured by moderate rather than severe depression.

Food organisms are captured only during the hours of daylight. *Evadne* appears to be a selective feeder the diet of which consists mainly of tintinnids and peridinians.

Several aspects of the biology of the species are discussed with reference to the findings on the food and feeding habits.

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EXPLANATION OF PLATE I

(Magnification, × 66.)

Evadne nordmanni.

Fig. 1. Primiparous female with early-stage embryos.

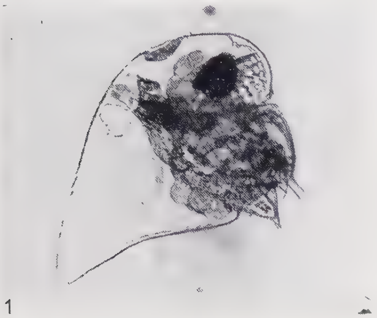
Fig. 2. Female with a single late-stage embryo.

Fig. 3. Female about to moult and liberate a single late-stage embryo. Early-stage embryos are present in the newly formed brood pouch.

Fig. 4. Female with a second or subsequent brood of early-stage embryos.

Fig. 5. Male.

Fig. 6. Female with an almost fully developed resting egg.



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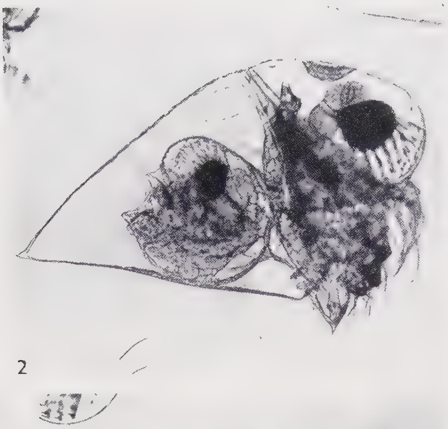

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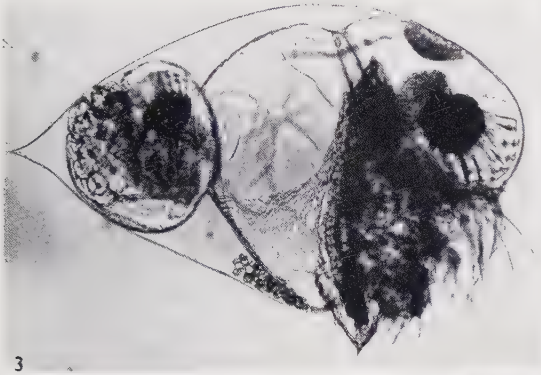

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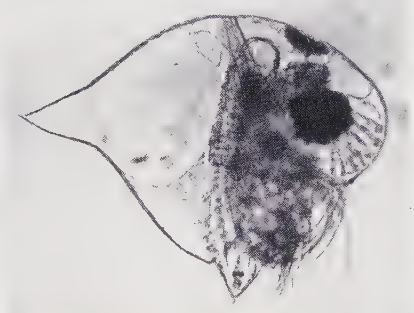

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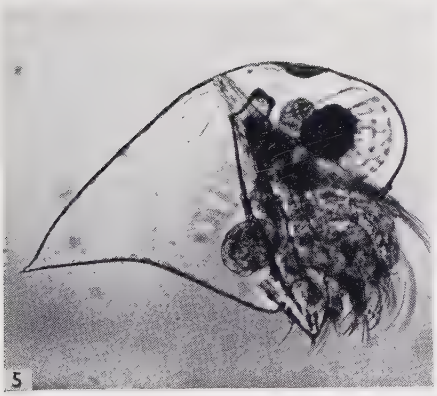

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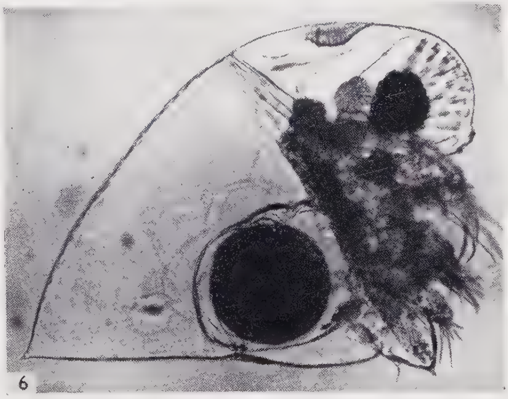

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J. mar. biol. Ass. U.K. (1958) 37, 371–377
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PHOSPHORUS AND SILICON IN SEA WATER OFF PLYMOUTH DURING 1956

By F. A. J. Armstrong

The Plymouth Laboratory

(Text-figs. 1–4)

Analyses of sea water collected during 1956 at the International Hydrographic Station E1 (lat. 50° 02′ N., long. 4° 22′ W.) are given here in the same form as in earlier reports (Armstrong, 1954, 1955, 1957). The methods of collection and analysis were substantially unchanged.

I am obliged to Lt.-Cdr. C. A. Hoodless and the crew of R.V. *Sarsia* and to Capt. W. J. Creese and the crew of R.V. *Sula* for assistance at sea. It is a pleasure to acknowledge the help given by Dr S. M. Marshall in making the counts of organisms listed in Table 3, and that of Dr T. Peirson, Medical Officer of Health for Plymouth, in providing access to the daily weather records of the city. Salinities were determined by the Government Chemists Department.

RESULTS

Temperature and salinity

The lowest surface temperature recorded was 8·7° C on 26 March, and the highest was 15·7° C on 23 July.

The vertical distribution of temperature during the year is shown in Fig. 1. It shows no sharp stratification until July; although on 11, 16 and 24 April there were small temperature gradients, the top of the water column being warmer than the bottom by 0·67, 0·28 and 1·12° C respectively. Temperature gradients then increased until July, when there was a sharp thermocline at 10–15 m, which persisted, with some variation in depth until September; it had broken down by 23 October.

Salinities throughout the year are given as integral means for the water column in Table 1. It is seen that there was a significant rise in March to 35·41‰ and that high values persisted until July. Such saline water is unusual at Station E1, figures of over 35·4 having been found only on six occasions out of sixty or so during the previous nine years. These unusual and persistent salinities give some grounds for supposing that during the spring months there may have been a consistent body of water at the station.

Phosphate

Vertical distribution is shown in Fig. 2, and integral mean concentrations in Table 1.

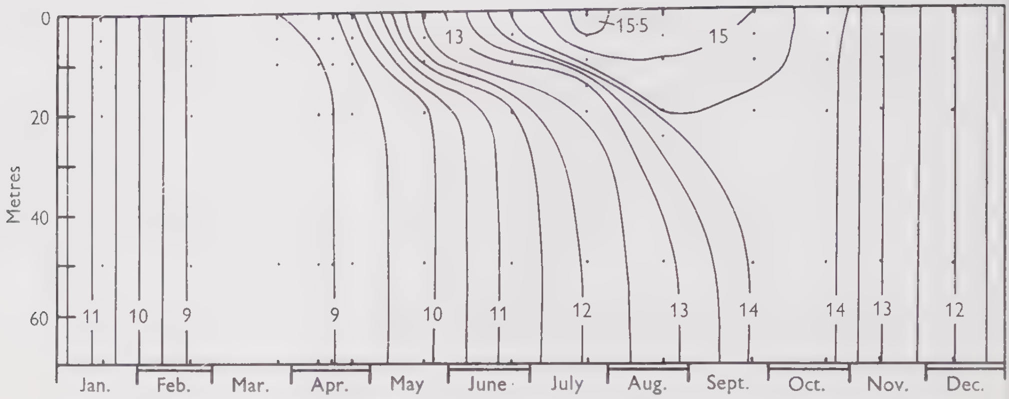

Fig. 1. Vertical temperature distribution at International Hydrographic Station E1, 1956. Contour lines at 0·5° C intervals.

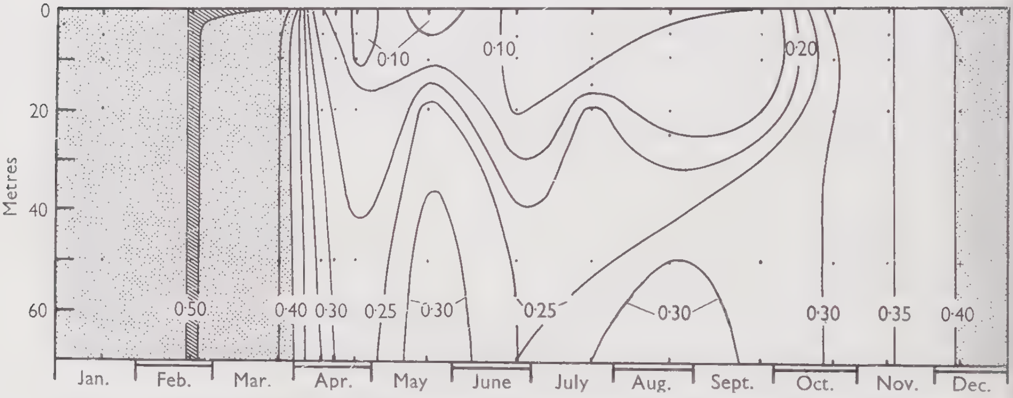

Fig. 2. Vertical distribution of phosphate at International Hydrographic Station E1, 1956. Contour lines at 0·05 μg atom P/l. intervals.

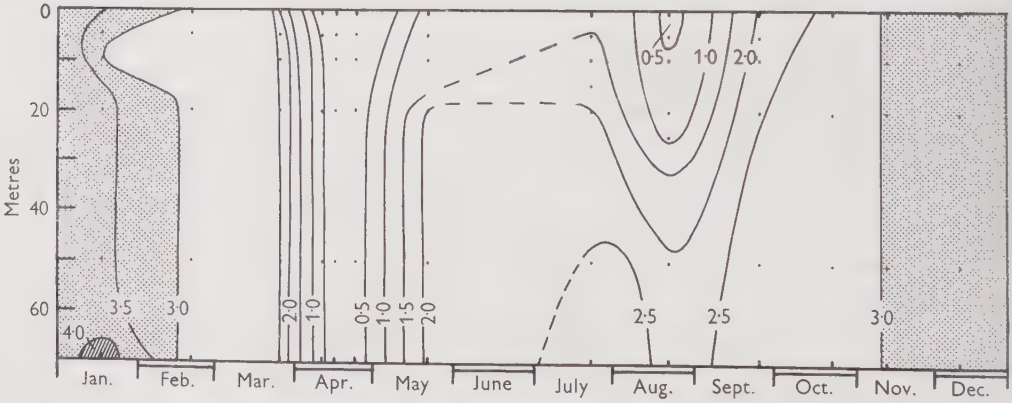

Fig. 3. Vertical distribution of silicate at International Hydrographic Station E1, 1956. Contour lines at 0·5 μg atom Si/l. intervals.

The maximum found was 0·50 μg atom P/l. on 21 February. This is about the usual value of recent years; the unusually high (0·58–0·59) ones of February and March 1955, were transitory. On 26 March, just after 2 days of bright weather, phosphate was 0·46 μg atom P/l. and phytoplankton was readily visible to the unaided eye in samples from all depths. Sunny anti-cyclonic weather continued, and while it lasted observations were repeated, on 11, 16 and 24 April, in order to follow chemical changes during the spring growth of plants.

TABLE 1. INTEGRAL MEAN CONCENTRATIONS IN WATER COLUMN AT STATION E1

Date	Salinity (‰)	Phosphate-P (μg atom P/l.)	'Total-P' (μg atom P/l.)	Silicate (μg atom Si/l.)
17. i. 56	35·27	0·46	0·59	3·61
21. ii. 56	35·32	0·50	0·60	2·87
26. iii. 56	35·41	0·46	0·58	2·52
11. iv. 56	35·43	0·25	0·51	0·61
16. iv. 56	35·42	0·20	0·49	0·08
24. iv. 56	35·43	0·17	0·50	0·27
22. v. 56	35·42	0·27	—	1·90
25. vi. 56	35·43	0·16	—	—
23. vii. 56	35·43	0·21	—	2·29
22. viii. 56	35·37	0·22	—	1·41
25. ix. 56	35·30	0·19	—	2·51
23. x. 56	35·30	0·31	—	2·72
13. xi. 56	35·34	0·34	—	3·05
10. xii. 56	35·37	0·41	—	3·08

TABLE 2. SUNSHINE ON PLYMOUTH HOE (22 MILES FROM STATION E1)

		1956		1893–1952, mean for 60 years	
Period	Days	Total sunshine (h)	Mean per day (h)	Total sunshine (h)	Mean per day (h)
26 Mar.–10 Apr.	16	111·1	6·9	81·5	5·1
11–15 Apr.	5	17·8	3·6	28·6	5·7
16–23 Apr.	8	87·6	10·9	53·6	6·7
Total for period	29	216·5	7·5	163·7	5·6

Table 2 shows total and mean daily hours of sunshine on Plymouth Hoe, about 22 miles from Station E1, for the period 26 March to 23 April 1956, and also the mean figures from the daily records, for the same period, for 60 years from 1893 to 1952. There was more sunshine in 1956. It was noticed that only 4 days (29 March and 12, 13 and 14 April) had less than 3 h of daily sunshine.

Integral mean concentrations fell to 0·17 μg atom P/l. during the period, the change being greatest in the upper layers and less marked below 20 m, as shown in Fig. 4. By themselves these phosphate observations are not particularly significant and resemble changes in earlier years. They are of interest, however, if taken together with the silicate analyses below.

Afterward, phosphate remained low in the upper layers (minimum 0·05–0·06 μg atom P/l. on 25 June) until September. By 23 October, when the water column had become isothermal, phosphate had also become uniform vertically, and increased until the end of the year.

Total phosphorus

Determinations were discontinued after April. The maximum integral mean value was 0·60 μg atom P/l. on 21 February. The March and April values show that to some extent the element is conserved during the spring outburst of plants.

Silicate

Vertical distribution is shown in Fig. 3, and integral mean concentrations in Table 1.

The maximum integral mean concentration of 3·6 μg atom Si/l. was found on 17 January, although the vertical distribution was not uniform, being 4·2 at 70 m and 3·0 at 10 m, increasing again to 3·5 at the surface. The surface was very slightly warmer (0·04° C) than the bottom. A decrease to 2·9 μg atom Si/l. had occurred by 21 February, temperatures then being uniform. Possibly the water mass was changing in January, though there is no other evidence for this.

Changes during the period 26 March to 24 April are shown, with the corresponding phosphate changes, in Fig. 4. During the first 21 days silicate decreased almost linearly, and on 16 April most of it had been removed from the water at all depths. Even at 50 and 70 m there remained only 0·08 and 0·09 μg atom Si/l. This is well below the photosynthetic zone, and the 'compensation point' as defined by Jenkin (1937) could hardly have been deeper than 40 m. After a further 8 days silicate, particularly in the deep water, had increased again somewhat, though phosphate had continued to fall.

During the summer months silicate concentrations fluctuated rather, mostly in the top 20 m. Vertical uniformity was re-established by 23 October, after which silicate increased slightly as is usual.

Integral mean concentrations

These are shown in Table 1 (and have already had mention). The decreases representing consumption of nutrients in the spring may be taken as: phosphate 0·34 μg atom P/l., and silicate probably 2·78 μg atom Si/l. if the January figures be excluded.

Plankton

Samples from 10 and 50 m taken on 16 and 24 April were preserved and were examined by Dr S. M. Marshall, who made duplicate counts of 10 ml. and gave the results listed in Table 3. She commented that only the 10 m

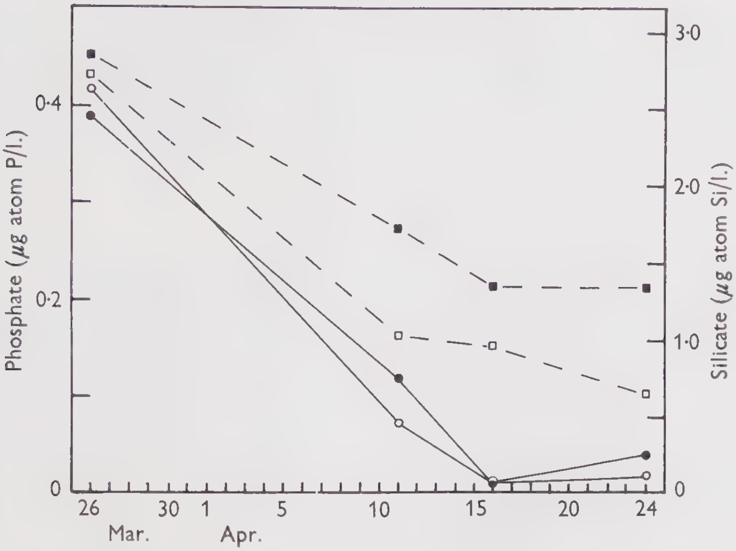

Fig. 4. Phosphate and silicate at 5 and 50 m at International Hydrographic Station E 1, 26 March to 24 April, 1956. Phosphate at 5 m, □; at 50 m, ■. Silicate at 5 m, ○; at 50 m, ●.

TABLE 3. ORGANISMS IN 20 ML SEA WATER CENTRIFUGED

April 1956. Station E 1

	16. iv. 56		24. iv. 56	
	10 m	50 m	10 m	50 m
Paralia sulcata	35	—	—	1
Coscinodiscus sp.	1	1	—	—
Thalassiosira sp.	2	2	—	15
Lauderia	3347	2720	980	3631
Stephanopyxis	5	7	1	13
Rhizosolenia				
Straight needle type	57	55	48	46
R. stolterforthii	77	119	30	126
Other *affines* types	108	57	43	114
Chaetoceros				
Phaeoceros	283	152	10	93
Hyalochaete	450	320	90	90
Biddulphia	3	—	—	—
Cerataulina	23	23	4	10
Eucampia	2	19	—	20
? *Navicula membranacea*	319	411	58	177
? Naviculid	2	2	1	1
Totals	4714	3888	1265	4337
Prorocentrum	—	—	1	1
Dinophysis and *Phalacroma*	1	1	6	—
Gymnodinian	—	—	2	—
Peridinium various spp.	13	11	28	20
Ceratium fusus	—	—	1	—
? Cyst	—	—	2	—
Distephanus	1	1	—	—
Tintinnid	—	—	—	4
Naked ciliate	—	—	1	—
Phaeocystis colonies	—	—	—	4
Temora nauplius	1	—	—	—
Oithona nauplius	1	—	—	—

Much detritus in all samples.

sample of 24 April differed much from the rest; it was certainly much lower in diatoms and probably higher in dinoflagellates, and that the numbers of *Lauderia* in the other three samples appeared not to be significantly different. There was also a distinct fall in the numbers of *Navicula membranacea* (identification not certain) and *Chaetoceros* between the dates.

DISCUSSION

The March and April analyses and the plankton counts call for some comment. It has been shown that these were found during 4 weeks of bright settled weather, when it may perhaps be assumed that little general change occurred in the water mass sampled. Usually, at this time of year, when the water begins to warm up after the winter, there is a good deal of vertical mixing both from above (wave-motion from spring gales) and from below (tidal drag on the bottom), which tends for a time to keep temperatures and nutrient concentrations uniform throughout the water column. However, in 1956 there was a temperature gradient with warmer water at the surface after 26 March, so that the resultant density gradient would have restricted that part of vertical mixing due to wave-motion turbulence. Indeed, in April, the phosphate figures also show a gradient, increasing toward the bottom. It is likely that less restricted vertical mixing took place between 11 and 16 April, which included three dull rather cold days with winds of force 5. The temperature gradient decreased, and so also did that of phosphate.

In these otherwise stable conditions silicate concentrations fell sharply, and by 16 April were very low, not only in the euphotic zone, but at the bottom. During 3 weeks the ratio of silicon to phosphorus removed from solution was 2·44:0·26 by atoms or just over 8:1 by weight. The silicon:phosphorus ratio in diatoms is found, by chemical analysis, in the range 16:1 to 50:1 (Vinogradov, 1953). Since the counts show that in size and numbers diatoms predominated over other organisms in the water, this suggests that they were deficient in silicon.

There seem to have been considerable numbers of diatoms in the deepest water; the counts show that this was so at 50 m. Some diatoms no doubt are carried out of the illuminated zone by turbulence. There is evidence too that at times they may lose buoyancy and sink rapidly (Marshall & Orr, 1928; Riley, 1941; Steele, 1956). Jenkins (1955) has shown that at Station E1, in March 1953, there was more chlorophyll in material filtered from bottom water than from the layers above. The counts of 24 April suggest either that diatoms in the upper water have been removed by some selective agency or that some have fallen into deeper water.

It is possible that diatoms in the deep water, out of the illuminated region, had continued to absorb silicate up to 16 April even if unable to make normal growth. Afterwards, many may have been eaten and voided by copepods, or may have died quickly, so that some silicate was returned to solution by 24 April.

The continued uptake of silicate, in the dark, by diatoms deficient in silicon, was observed by Lewin (1954), with cultures of *Navicula pelliculosa*.

SUMMARY

The results of analysis of sea water from the International Hydrographic Station E1 during 1956 are presented in graphical form and as integral mean values for the water column of 70 m. The seasonal variation is shown; it appears that consumption of phosphate in the spring outburst of plants was 0·34 μg atom P/l., and that of silicate 2·78 μg atom Si/l. The spring outburst was followed more closely than usual in the period 26 March to 24 April during 4 weeks of rather bright weather. Nearly all the silicate in the water was taken up, and some phosphate left, and it seems that the rapidly growing diatoms, which predominated at the time, were deficient in silicon. It is suggested that in the deep water they continued to absorb silicate, although not receiving enough light for growth and division.

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FURTHER OBSERVATIONS ON PROPRIOCEPTORS IN CRUSTACEA AND A HYPOTHESIS ABOUT THEIR FUNCTION

By J. S. Alexandrowicz
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(With Plate I and Text-figs. 1–5)

The observations on proprioceptors in the legs of Crustacea recorded recently (Alexandrowicz & Whitear, 1957) have shown that in these animals there are several receptor organs in the coxal region and that there is a difference in their number in the thoracico-coxal articulation. Whereas in some species, e.g. *Homarus* and *Astacus*, there are two receptor units running side by side, termed for short muscular and elastic receptor, in others, e.g. the Brachyura, only one, of muscular type, is present. It was stated, moreover, that in the latter case the arrangement of the sensory elements of this receptor exhibit certain features differing from those in the organ of the *Homarus* type. But the question was left open whether this particular pattern of innervation is an inherent feature of the muscular receptor when it is not associated with a second organ, and, if this is so, what inference concerning its mode of function could be deduced therefrom. It seemed, therefore, worth while to investigate the receptors in such species as *Eupagurus bernhardus* and *Palinurus vulgaris* in which, as some preliminary observations had shown, only one receptor unit is present. In the present paper are reported the results obtained with *Eupagurus*, which had the advantage of being easily obtainable.

METHOD

The nerves were stained with methylene blue and the method used was the same as previously recorded. The organs in *Eupagurus* stain readily so that a complete failure is never experienced, although the staining, as is usual with this method, is uneven. The main difficulty consists in exposing the organs in such a way that the methylene-blue solution can have free access to them and to keep all the parts of the preparation in the desired position. The usual method is to attach them to paraffin plates. Paraffin wax, however, has the disadvantage of being soluble in xylol. This is of no importance when the preparations are of such size and shape that after being stained and fixed they can be detached from the plates and remain flat up to the final stage of mounting. When, however, such objects as fine fibres pass through alcohol and xylol they usually become bent or deformed in various ways. In handling such preparations polyethylene ('polythene') plates proved very serviceable, since the tissues attached to them with the finest pins can be transferred into xylol and remain perfectly stretched in it. I am indebted to Mr F. A. J. Armstrong for this suggestion and for providing the plates. This method can be also used when embedding easily deformable objects; they can pass unaltered from the moment of fixation through all media, to be detached only before being transferred into the paraffin bath or even shortly before embedding. Polyethylene, however, is not yet an ideal material for similar purposes since it is affected by xylol, although very slightly. It is to be hoped that with so many kinds of plastics now being manufactured, or being invented, some of them will prove to be satisfactory in every respect.

OBSERVATIONS

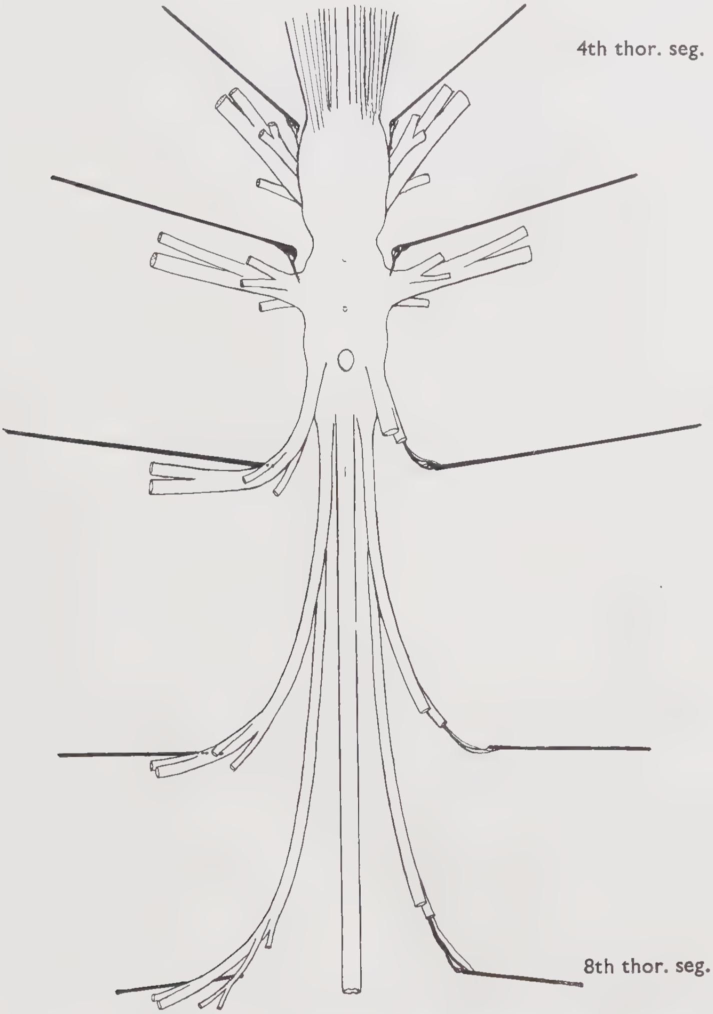
In the coxal region of *Eupagurus bernhardus* the following receptor organs have been found: (i) the muscular receptor spanning the thoracico-coxal articulation; (ii) two innervated strands associated with the muscles levator and depressor basipoditis; and (iii) the coxo-basipodite receptor.

Muscular receptors

The muscular receptors are present, one on each side, in all five peraeopods. They have their proximal attachments in the vicinity of the ganglionic cord, but their position in relation to this cord and its main nerve trunks is different in each segment owing to the coalescence of the thoracic ganglia and various directions of the course of the trunks (Text-fig. 1). In the 4th–6th thoracic segments the receptor muscle has its origin on the posterior surface of the endosternite plate limiting anteriorly the respective segments. In the 4th segment it is near the border of the ganglionic mass and ventral to it; in the 5th segment, in which it is more easily accessible, it is situated laterally to the ganglion and in front of the main nerve trunks (Pl. I, fig. 1); in the 6th segment it is covered by thick nerves of the limb. In the 7th and 8th segments, in which the endophragmal skeleton is greatly reduced, the receptor muscles originate on small chitinous plates and are also covered by main nerve trunks running to the legs.

The endosternite plates do not exhibit any special projections at the point where the muscles arise. In this respect *Eupagurus* differs from all decapods examined in which special receptor rods are present in several segments (Macrura) or at least in one of them (Brachyura).

At the proximal end of the muscle its myofibrils pass into fine, short (*ca.* 100 μ), connective tissue fibres which thus constitute a tendon by means of which the receptor is attached to the membrane lining the calcified chitin of the endosternite. Despite its insignificant appearance this tendon, as will be discussed later, plays an important role. From this point of origin the muscle runs in the direction of the anterior hinge of the basipodite to insert near the rim of the coxa. The muscle fibrils end here without passing into a tendon like that at their proximal attachment. They do not reach the calcified chitin of the coxa but insert near to it into the tissue of the articulation membrane. Alongside the muscle run connective tissue fibres which form two wider strands proximally, as in the Brachyura. Near the distal end of the muscle some of the fibres deviate sidewards to pass into the membrane of the articulation (Pl. I, fig. 3).



Text-fig. 1. *Eupagurus bernhardus*. Topography of the thoracico-coxal (muscular) receptors in relation to the ganglionic cord and its main nerve trunks seen from the dorsal side. On the right side parts of the nerve trunks covering the origin of the receptors have been removed.

Nerves

Motor nerves.

The motor fibres innervating the muscle run near the sensory nerves but do not associate with them more closely and often, especially in the posterior segments, are distinctly separated from them (Pl. I, fig. 4, *mot.*). The distribution of their branches follows the same pattern as in the receptors of other crustaceans.

Sensory nerves.

The appearance of the sensory innervation of the receptor in a well-stained preparation gives the impression that in *Eupagurus* these nerves are much more numerous than in other species (Pl. I, fig. 4). On closer scrutiny this abundance proves to be due to branching of the main fibres at a greater distance from the muscle and the real number of these fibres, i.e. the number of neurons emitting them, is most probably the same as in crabs. The following elements can be distinguished: (1) two thick fibres; (2) one thinner fibre; and (3) an uncertain number of fine ones. All these fibres run in a common bundle which in the 4th and 5th thoracic segments (of the 1st and 2nd peraepod) can be followed up to the ganglion as an individual trunk (Pl. I, fig. 1). In the 6th to 8th segments in which the nerves have a longer way to travel to the ganglionic cord they associate with the nerves of the legs (Text-fig. 1).

The two thick sensory fibres of the receptor have different designations. One of them, fibre *T* (Text-fig. 2), runs as in the Brachyura towards the point of origin of the muscle and divides into three or four stout branches which with their very short arborizations end on its tendon. The smallness of the area of distribution of fibre *T* is particularly striking considering its diameter which attains 40 μ or even more (Pl. I, fig. 2).

The second fibre *S* (Text-fig. 2) divides into two main branches which innervate the strands flanking the muscle. When, as often happens, this fibre splits into many branches before reaching the muscle, the branches range themselves into two batches each passing on to one of the strands; in other instances the two thick fibres penetrate into the strands and branch afterwards. Many variations in the arrangement of these fibres can be met with, and even on the opposite sides of the same segment the picture of their distribution can look quite different. The fibres dividing in the strands can be followed for some distance down the muscle, but how far they extend is uncertain.

The thinner fibre *P*[1] divides into several branches which pass on to the same strands as those of the fibre *S*, but do not seem to extend so far distally as the latter. Their endings are not so clearly delimited as in crabs, but there is ground for assuming that their areas of termination are independent of those of the branches of fibre *S*.

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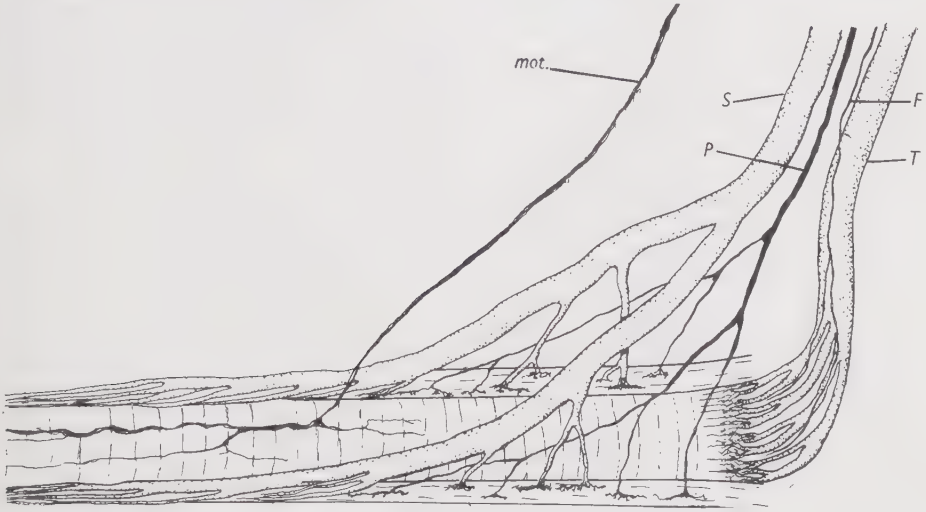
[1] For discrimination of the various elements their designation by letters is adopted. The fibre ending on the tendon is called fibre *T*, that ending on the strands, fibre *S*. The element designated now as fibre *P* was in the previous paper referred to as 'thin fibre'; this term does not seem to be appropriate as this fibre is not very thin and there are others which are thinner, called now fibres *F*. Such terms as 'accessory' or 'small' fibres have been avoided as they have been applied to definite elements in other instances.

The most elusive elements are the fine fibres *F* which can sometimes be seen running with the fibre *T*, as has also been noticed in other species. They are here distinguished as a special sort, but the possibility that they are of the same kind as the fibre *P* or even branches of the same neuron is not excluded. As always when many nerves run side by side, there are many doubts whether a given fibre is an individual element or is only a branch of another fibre travelling in the same bundle.



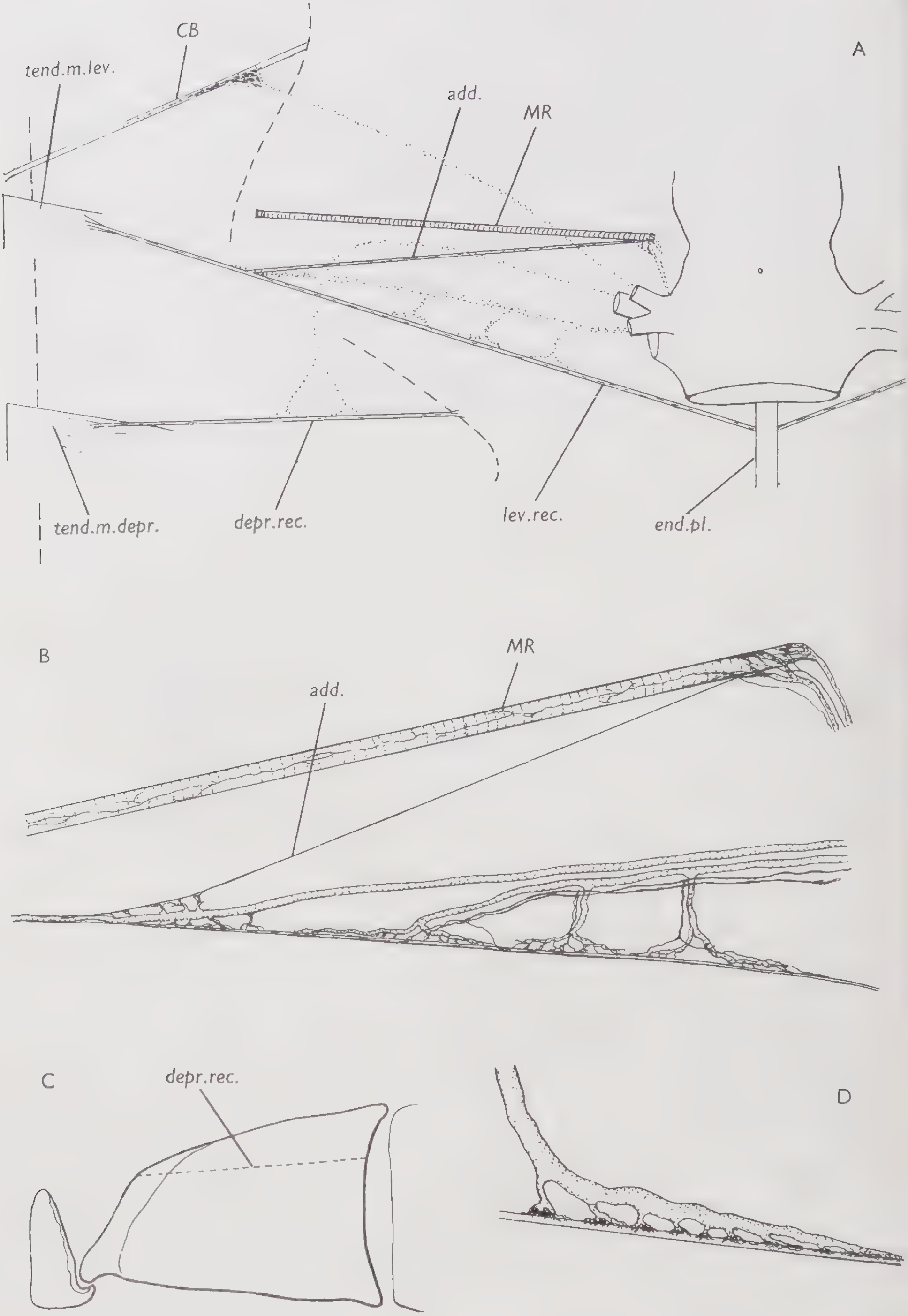
Text-fig. 2. *Eupagurus bernhardus*. Proximal part of a thoracico-coxal receptor. *T*, fibre ending on the tendon of the receptor muscle; *S*, *P*, fibres innervating the strands flanking the muscle; *F*, fine fibres accompanying the fibre *T*; *mot.*, motor nerves of the receptor muscle.

Innervated strands

Organs of a different category, such as have been described in *Homarus* and Brachyura under the name of levator and depressor receptors, have also been found in *Eupagurus*. Their general disposition shows certain differences in this species, but they have similar relations to the muscles of the basipodite which gave rise to their designation. Each of these organs consists of a fine strand of connective tissue having elastic properties and nerve branches ending on it.

Levator receptor

The proximal attachments of the levator receptors are not the same in all segments. In the 4th segment the strand arises on the anterior surface of the endosternite plate separating the 4th and 5th segment. In the 5th and 6th segments it arises on the plates of the endophragmal skeleton projecting dorsally in the


A
CB
tend.m.lev.
add.
MR
tend.m.depr.
depr.rec.
lev.rec.
end.pl.
B
MR
add.
C
depr.rec.
D

Text-fig. 3 For legend see opposite page.

mid-line in each of the 4th to 6th thoracic segments; the points of attachments of the strands of the opposite sides lie thus very near to each other (Text-fig. 3 A). In the two posterior legs they are again at a distance from one another.

In all legs the strand runs on the bundles of the ventral portion of the m. levator basipoditis near to its anterior margin. Approaching the tendon of the muscle it splits into very fine twigs which insert on the tendon between the muscle bundles.

In the distal half of its course this strand is joined by an additional strand which arises close to the muscular receptor and, after crossing obliquely the nerve trunks of the limb, unites with the main strand at an acute angle (Text-fig. 3 A, B; Pl. I, fig. 7). The length of the main strand in the 2nd and 3rd peraeopod of a larger specimen is about 12 mm and its diameter varies from about 30 μ in the middle part to some 10 μ and less towards its distal end. The additional strand has a more uniform thickness in the range of 5–10 μ.

The main strand is supplied by no less than three and often four nerve branches given off by a nerve trunk which also carries motor fibres for the ventral portion of m. levator. One of the nerve branches reaches the strand always at the point where it is joined by the additional strand and the other three proximally to it and at a distance from one another (Text-fig. 3 B; Pl. I, figs. 5, 7). The three proximal branches (or two if there are three in all) consist of one thick and one or more thin fibres (Text-fig. 3 B, Pl. I, fig. 8); some of the thin fibres can sometimes be seen to run separately to the strand. In the most distal branch the thin fibres have not been noticed. The number of neurons taking part in this innervation could not be ascertained as the fibres run towards the ganglion in a common bundle with other nerves; it seems probable that the thick fibres belong to two neurons; as to the thinner it can only be said that they appear to be emitted by more than one neuron.

The photographs (Pl. I, figs. 7, 8) give an idea of the striking disproportion between the calibre of the nerve fibres (*ca.* 50 μ) and that of the strand on to which they pass not far from one another. They give off short branches which almost immediately resolve into numerous arborizations (Text-fig. 3 D). Of the details of their terminations little can be seen with the ordinary microscope: in methylene-blue preparations they show irregular outlines and appear

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Legend Text-fig. 3

Eupagurus bernhardus. (A) Diagram of the proprioceptors in the coxal region. *MR*, thoracico-coxal (muscular receptor); *lev.rec.*, *depr.rec.*, levator and depressor receptors; *CB*, coxo-basipodite receptor; *end.pl.*, plate of the endophragmal skeleton; *tend.m.lev.*, *tend.m.depr.*, tendons of the muscles levator and depressor. The nerves are drawn in dotted lines and the interrupted lines indicate the margins of the coxopodite. (B) Innervation of a levator receptor of the left side. Three of its nerve branches are represented as belonging to one neuron and the fourth, the most distal one, to a second neuron (the most probable disposition); *add.*, additional strand with its proximal attachment at the origin of the muscular receptor *MR*. (C) Coxopodite of the 2nd left peraeopod from below showing the projection of the depressor receptor *depr.rec.* (D) Mode of branching of a thick nerve fibre on the strand of a levator (or depressor) receptor.

confluent with one another. It is to be hoped that examination with the electron microscope for which these organs seem to be a particularly suitable object will show interesting features of the relations of the nerve fibres to each other and to the tissue of the strand.

To the question how much of the whole length of the strand is innervated no satisfactory answer can be given. The fibre entering the angle between the two strands can be followed for some distance until it fades out; it seems probable that the regions near the attachments have no innervation, but where its limit is is uncertain.

The additional strand is supplied by very short branches of the nerve fibre near its point of junction with the main strand (Text-fig. 3B) and no other nerves in the additional strand have been noticed. It may be recalled that in *Homarus* the additional strand is innervated by a fibre entering its proximal end. No such fibre could be seen in *Eupagurus*; if there is one it could be only a fine filament which does not show in my preparations.

The levator receptors of the two posterior legs are very tiny elements and it is more difficult to expose them without damaging some of their parts. That of the 4th leg has an additional strand while in the 5th leg this appears to be missing. The number of nerve branches in the 5th leg seems to be reduced to three or two.

Depressor receptor

The depressor receptor has been found in the three anterior peraeopods; whether or not it is present in the two posterior ones is a doubtful point. It is situated at the ventral wall of the coxal segment and extends from the proximal border of the coxa to the tendon of m. depressor basipoditis into which it inserts not far from its distal end. The position of its strand seen by transparency is shown in Text-fig. 3C. It runs near the anterior border of the short head of the depressor muscle arising on the rim of the coxa. Close to the bundles of this muscle is the attachment of the receptor strand the fibres of which pass here on to the tissue continuous with the membrane of the coxal articulation. It should be added that the short head of the depressor muscle is covered by longer bundles of another portion of the depressor muscle originating on the endophragmal skeleton; the receptor strand is therefore situated under these longer bundles.

The nerve carrying the fibres of this receptor is one of the branches innervating the depressor muscle. The branch of the receptor runs near the antero-ventral wall of the coxa and usually bifurcates at a distance from the strand (Pl. I, fig. 6). The two branches of this division, each containing two fibres of different calibre, pass on to the strand and behave in the same way as in the levator receptor. No adequate information about the number of fibres taking part in this innervation could be obtained; it is, however, evident that this organ has a poorer innervation than the levator receptor.

Coxo-basipodite receptor

The coxo-basipodite receptor is present in all five peraeopods. It consists of a connective tissue strand and numerous bipolar nerve cells ending on the fibres of the strand. It looks exactly like those described in *Homarus* and its position is the same, viz. it originates near the dorsal hinge of the coxa and runs towards the basipodite to insert near the tendon of m. levator (*CB*, Text-fig. 3 A). The axons of the cells unite into a bundle joining the nerve which arises from the ganglion dorsally from the thickest nerve trunk of the limb and which supplies the dorsal part of the coxa.

The disposition of all receptor organs in the coxal region is shown in Text-fig. 3 A. Their longitudinal dimensions and their situation in relation to the mid-line of the body are drawn to scale, but they are represented diagrammatically as if lying in one plane while in fact they occupy different positions in relation to the axis of the leg.

Hints on dissection

The proprioceptors in the coxal region have to be exposed from the dorsal side. After removing the carapace and the digestive organs, the dorsal parts of the endophragmal skeleton and the muscles should be cut away until the ganglionic cord becomes exposed. The muscles covering the nerve trunks in the coxa should be removed, care being taken not to damage the ventral portion of m. levator on which lies the levator receptor. It is not advisable to do much cleaning of the organs until the staining makes the tissues better visible. The difficulties in finding the muscular receptor in *Eupagurus* are aggravated by their topography, which differs in each segment. That of the 2nd peraeopod is better taken first because it is more easily accessible. Once the organ is spotted one can proceed further with cutting out parts of the muscles, removing the blood clots, arteries, etc., but all this work must be done by stages.

The receptor of the chela is less accessible because it lies more ventral and nearer to the ganglionic mass. The organs in the 3rd to 5th legs are covered by nerves and these must be pulled aside or cut out if the receptors are to be exposed. The frequent mistakes can be corrected when dissecting the opposite leg. The muscular receptor of the 5th leg, although apparently most hidden of all, proved to be quite convenient for staining and all other manipulations, as the preparation can be more easily spread and pinned down to paraffin or polyethylene plate than those from other segments. To reach the receptor all the overlying muscles must be removed until the big nerve trunks are free. Beneath them, as shown in Text-fig. 1, is the origin of the muscle. In male animals this point lies directly in front of and not far from the end portion of the vas deferens. The receptor muscle runs close to the anterior bundles of a thin layer of the ventral portion of m. depressor. To expose it parts of the nerves as well as the artery covering it must be removed. When the receptor is spotted it can be freed from overlying tissues along its whole length, which is 4 mm in the largest specimens.

The levator receptor can easily be found in the 2nd and 3rd peraeopod provided it has not been torn away during dissection. It can be located even before the strand itself becomes visible by the characteristic branching of its nerves. These nerves stain readily but their stain fades out in a comparatively short time.

To find the depressor receptor it is necessary to remove the muscles lying dorsal to that part of the coxa where the receptor strand is attached (Text-fig. 3c); great care must be taken not to damage it when cutting out the muscles in that region. It is therefore better when arriving near the receptor to pull the muscles aside, fix them with pins and observe the progress of staining until the receptor can be distinguished.

To obtain preparations of the coxo-basipodite receptor the dorsal parts of the coxa and of the basipodite should be cut out and the adhering muscles pulled aside so that the staining solution has access to the region in which this receptor is situated. When it is visible, the unnecessary tissues can be cut out to expose it better. It is also possible to get preparations with this organ *in situ* still connected with the central nervous system. For this purpose, in a preparation made as for staining of the muscular receptor, the posterior part of the coxa and of the basipodite should be removed and their anterior parts held in such a position that the progress of the staining of the coxo-basipodite receptor can be followed.

Comparison of the proprioceptors in Eupagurus *with those in other Crustacea*

The observations described above have shown that the proprioceptors in the coxal region of *Eupagurus* occur in the same number as in *Carcinus* and *Cancer*, and that they have similar histological structures the most remarkable feature of which is the location of the cell bodies of sensory neurons in the thoracic ganglia. The muscular receptor proved to have the same nerve elements as those in the two crabs and their disposition is basically the same; the differences in the general appearance of innervation is due to the mode of branching of nerve fibres and also to the fact that one of the elements ending on the strands flanking the muscle, the thinner fibre *P*, appears to have in *Eupagurus* a greater share in innervation, for it has more numerous and longer branches than in *Carcinus*. Major differences have been found in the topographical relations of the muscular, levator and depressor receptors, the origins of which in crabs are close together, while in *Eupagurus* they lie far apart. A complete isolation of the depressor receptor, as in *Eupagurus*, has not been hitherto seen in other species in which it has either a common attachment with the muscular receptor (*Carcinus*, *Cancer*, *Palinurus*), or is connected with it by an additional strand (*Homarus*). The levator receptor in *Eupagurus* resembles this organ in *Homarus* and *Palinurus* in that it has a main and an additional strand, but such disposition of nerves as is found in *Eupagurus*, viz. with four branches approaching the strand at a distance from one another, has not been observed before in any species.

THE PROBLEM OF FUNCTION OF THE RECEPTORS IN THE COXAL REGION

The elucidation of the functions of the receptor organs lies in the field of physiology, and hypothetical speculations about them can easily go astray if not verified by experiments. However, these organs in the legs of the Crustacea exhibit such remarkable structural differences that it seems worth while to analyse those features of their anatomy from which their function may be inferred.

Summing up all evidence available, the following categories of proprioceptors in the legs of Crustacea can be distinguished: (1) rows of nerve cells ending on connective tissue strands; (2) muscular receptor spanning the thoracico-coxal articulation; (3) innervated strands associated with the levator and depressor muscles; (4) Barth's organ.

All these receptors are in all probability concerned with the movements of leg segments, with the possible exception of the organ described by Barth (1934), the function of which is obscure and will not be discussed here. Some of them may, moreover, be responsible for the reflex of the compensatory eye movements (Dijkgraaf, 1956).

The organs of the first category, of which there are at least seven in each peraeopod may show some differences in the arrangement of their elements, but all are made on the same principle. They are evidently stretch receptors, and being situated at the articulations are presumably designed to register the position of the adjoining segments in relation to one another. The problem why in some joints there is only one organ and in others—e.g. mero-carpopodite and carpo-propodite joints—there are two, and what are their different functions, awaits solution. In view of the ubiquity of these organs and the relation of some of them to the muscles, the hypothesis of Burke (1954) attributing to this type of receptor in the propo-dactylopodite articulation the additional function of perception of vibrations is open to doubt.

Muscular receptor

The muscular receptor in the thoracico-coxal articulation, which consists chiefly of contractile substance, must work on a different principle. In trying to understand its function the fact should be taken into consideration that it can occur either alone, or accompanied by a second receptor which by its structure belongs to the first category mentioned above.

Let us first examine the latter type in which, as in *Homarus*, two receptors run side by side: the one, a muscle with sensory elements ending on its tendon, and the other, a strand of connective tissue fibres with many sensory nerve cells ending on them. In the former, stimulation of sensory endings can evidently be brought about by the pulling on the tendon, and it must therefore take place each time that the muscle contracts and its myofibrils draw on the tendon.[1] The contraction of this muscle occurs in all probability when the leg is moved forwards (Text-fig. 4A). This can be assumed from its position on the antero-ventral wall of the coxa in the vicinity of m. promotor coxae the name of which indicates its action. It has been moreover observed in some instances that the same fibre which takes part in the motor innervation of the receptor muscle sends its branches to m. promotor indicating the simultaneity of action of these two muscles.

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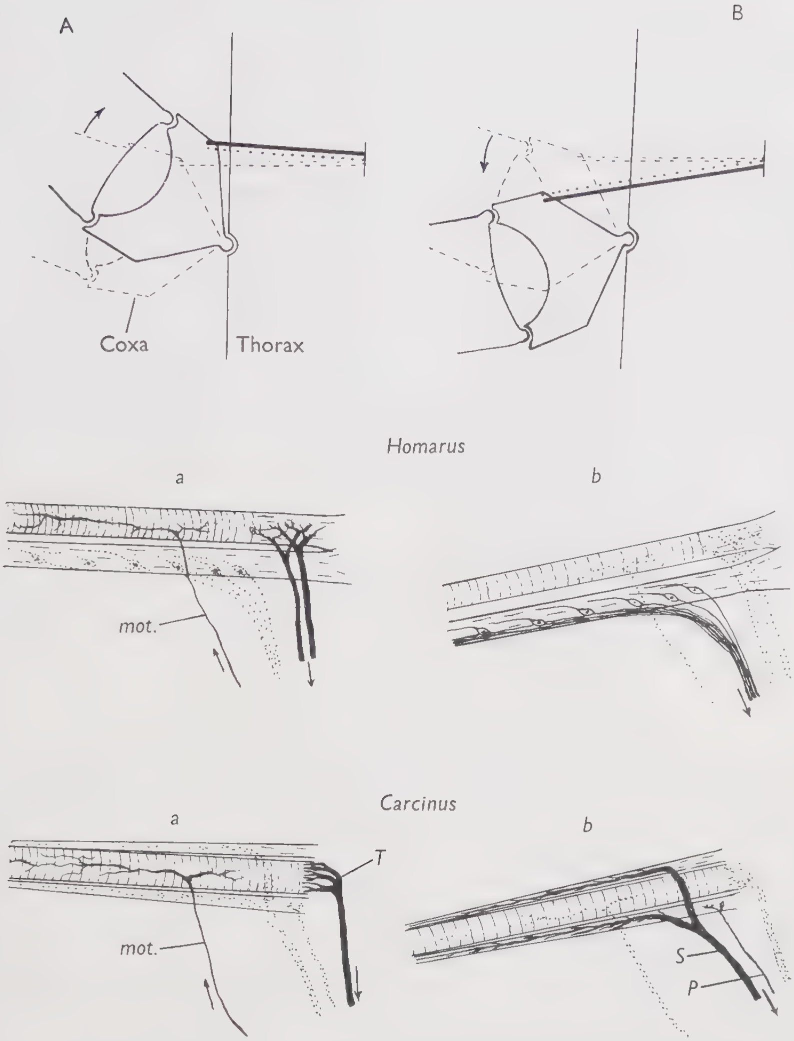
[1] It appears to be a general rule that the sensory elements in all the receptors known in Crustacea end not on the muscle itself, but on the connective tissue fibres. In contradiction to this Florey & Florey (1955) stated that in the abdominal receptor organs in *Astacus fluviatilis* no tendinous region is present and that the processes of sensory cells end directly on the muscle. This is a mistake. These organs in *Astacus* have intercalated tendons at the same place as in *Homarus* and *Palinurus*, i.e. where the nerve cells distribute their arborizations. Only a small number of myofibril bundles pass uninterrupted through this region in the same way as they are known to do in some segments of *Homarus*.

The second receptor, which runs close to the muscular receptor and has similar attachments, consists of non-contractile elements and consequently can respond only when being passively extended. As can be easily demonstrated, its stretching occurs during the backward motion of the leg (Text-fig. 4B). It may be therefore conjectured that the two receptors respond to the movements of the leg in the horizontal plane the muscular receptor being stimulated when the leg is moved forwards and the elastic receptor when it is pulled backwards (Text-fig. 4, *Homarus a*, *b*).

It cannot be overlooked that when the leg is moved backwards the distance between both ends of the muscular receptor is increasing and its tendon might be affected by the passive stretching of the muscle. It is, however, conceivable that this muscle may have a certain degree of slackness so that the pull on the tendon in the backward position is negligible and unable to stimulate its nerves. This view seems to be more probable than the assumption that the two organs are stimulated at one and the same time.

When the muscular receptor alone is present it is found to have a special arrangement of its nerve elements which may be called the *Carcinus* type, as it was first observed in this species; besides, being less complicated as in *Eupagurus*, it is also more suitable for diagrammatic representation. The most remarkable feature of this arrangement is the separation of the areas of termination of the two main sensory fibres. One of them, fibre *T* (Text-fig. 4, *Carcinus a*), clearly shows that it is destined to respond to contractions of the muscle and should therefore be regarded as conveying impulses during the forward motion of the leg. There is no reason to suppose that the second fibre *S* is affected at the same time for it passes into the strands flanking the muscles which have proximally only a loose relation with it and which in any event would be slackened when the muscle contracts. When, however, the leg moves backwards and the receptor becomes extended these strands must take up the strain and their stretching stimulates the endings of fibres *S*, *P* (Text-fig. 4, *Carcinus b*). It must be assumed that here too the muscle is so adjusted that during the backward movements of the leg no response is elicited in its fibre *T*. It may be recalled that the flanking strands pass distally into the sheath surrounding the muscle and thus can give protection to the latter.

If this interpretation is correct the receptor of this type would be a remarkably effective organ capable of controlling the actions of two antagonistic muscles, viz. mm. promotor and remotor coxae. It would represent a higher stage of evolutionary development, in which only one organ is present with all its sensory neurons having their cell bodies within the central nervous



Text-fig. 4. (A, B) Diagrams of the movements of the legs in the thoracico-coxal articulation. The axis of these movements is represented as being perpendicular to the plane of the drawing and that of the coxo-basal articulation in this plane (actually these axes in different species can be inclined at various angles to the horizontal and vertical planes and can more or less deviate from being at right angles to one another). The drawings of the proximal parts of the thoracico-coxal receptors of *Homarus* type and *Carcinus* type show in *a* and *b* the nerve elements entering into action during the movements indicated in the diagram above in the same column. The elements out of action are drawn in dotted lines. The two strands flanking the receptor muscle in *Carcinus* are represented in diagrams by one line only.

system, than in *Homarus* in which the same functions are performed by two organs one of which is of a primitive type with ordinary sensory cells situated outside the central nervous system.

It would be interesting to see whether intermediate stages in the evolution of receptors of the *Homarus* type to those of the *Carcinus* type can be found in other groups of Crustacea. One might suspect that in *Homarus* itself this evolution is at an early stage. The presence of two thick sensory fibres in its muscular receptor is suggestive in this respect. In the above considerations no account has been taken of differentiation in their function since in view of the abundance of their intermingling arborizations no distinct difference in localization of their endings could be ascertained. It is, however, not impossible that one of these elements has a tendency to distribute its branches on the more superficially situated connective tissue fibres and to pass farther in the distal direction. Conceivably, in the course of evolution, with the development of stronger strands alongside the muscle, this element has become the fibre *S* responding to stretching of these strands and has supplanted the less efficient receptor of the older type which thus became obsolete and disappeared.

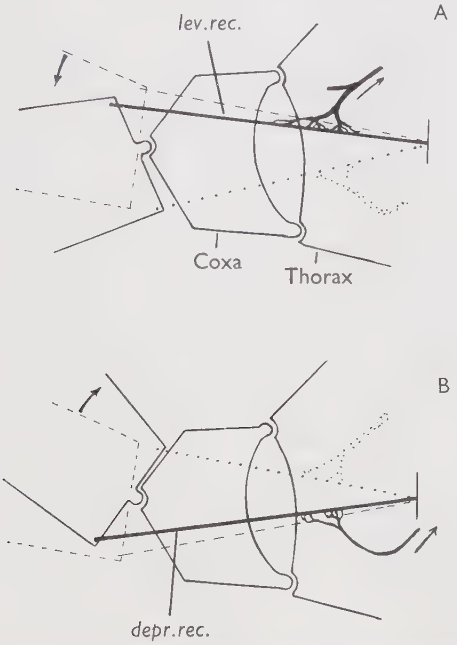
It is worth mentioning that in two instances, one somewhat doubtful but the other quite certain, I have noticed a small bipolar nerve cell among the nerve fibres of the muscular receptor in *Carcinus*. Such occasional cells might be vestiges of the primitive second receptor.

Innervated strands (*levator and depressor receptors*)

The levator and depressor receptors show differences in their arrangement as far as their proximal attachments are concerned. Their distal insertions, however, in all species examined, are associated with the insertions of the mm. levator and depressor respectively. On the assumption that the nerves of these receptors are stimulated when the strands on which they end become stretched, it is essential to determine in which positions of the leg segments this stretching must take place.

The two muscles (depressor and levator basipoditis) are antagonistic in their action. The contraction of the depressor pulls the basipodite and with it the whole leg downwards and when the legs are pressed against the ground the animal is able to lift its body. In this position of the basipodite, as shown in diagram (Text-fig. 5A), the dorsally situated strand of the levator receptor must become extended, and consequently whenever the animal stands up and walks its levator receptors are likely to be in action.

The ventral strand, inserting on the tendon of the depressor muscle, will become relaxed when the latter contracts, but is extended when the basipodite is pulled upwards (Text-fig. 5B). It is, however, not so obvious as with the levator receptor when precisely during this movement the receptor nerves can be stimulated, because when the body is actively lifted the ventral strand is more slackened, compared with its state in the resting animal, and from this state it is unlikely to respond immediately to the contraction of m. levator. It should, however, be borne in mind that as the animal walks the leg movements in the dorso-ventral plane are combined with those in the horizontal plane, and as the movements in the latter may exert some tension on the receptor strands they can contribute in bringing the leg segments into such a position that the depressor receptor can come into play. Thus, according to this interpretation the two innervated strands would be designed to regulate the action of the depressor and levator muscles, and it should be noted that the *levator* receptor would respond to the contraction of the *depressor* muscle and vice versa. The terms designating these organs relate therefore to their topographical connexions and not to their function. It is not proposed to change these names since they have been used in the description given previously, and moreover their anatomical relation is a fact whereas the assumptions concerning their function are as yet hypothetical.



Text-fig. 5. Diagram of the movements of the legs in the coxo-basal articulation. The plane of this drawing is at right angles to that in Text-fig. 4. The position of the basipodite in the resting animal is indicated by interrupted lines. (A) Contraction of m. depressor basipoditis. (B) Contraction of m. levator basipoditis.

Other hypotheses could also be put forward, as, for instance, that each of these receptors responds to variations of contraction of that muscle with which it is associated. There are two objections to this idea: first it implies that the stimulation of the receptors would not occur when the receptor strand is stretched but when it is slackened, which seems less likely; and secondly, it would not explain why the levator receptor has a richer innervation and in some species like *Eupagurus* and *Palinurus* appears to be a much more conspicuous organ. This feature fits the conception that the levator receptor controls the depressor muscle which has the important function of keeping the body above the ground as long as the animal is on the move, while the task of the levator muscle is only to lift the leg and keep it lifted when it is being shifted forwards.

It is true that the levator has an additional function, for it is the muscle which autotomizes the leg. The innervated strand ending with this muscle might thus be thought to have something to do with the autotomy. It is, however, difficult to imagine in what way it could take part in this process.

The two receptors in question appear to be better developed in some species than in others, possibly in relation to the degree of agility of the animals. In fact they are very well developed in such lively species as *Eupagurus* and *Carcinus*: in *Cancer pagurus*, a slowly moving crab, they appear to be less developed than in *Carcinus*, while in the sluggish *Maia* they are apparently absent altogether. Whether this is a general rule can only be established by a special study of more species.

To sum up, it would appear from the above considerations that every possible movement in the thoracico-coxal and coxo-basal articulations elicit stimuli in some of these receptors which transmit impulses to the central nervous system. And yet there is one more organ, the coxo-basipodite receptor, which is present in all peraeopods and must also play some role. Being made of connective tissue fibres and numerous nerve cells, it belongs according to its structure to the same category as the receptors in all other joints. Since the function of the latter is most probably to register the position of the segments, so its function is likely to be the same. Its apparently superfluous occurrence in the same articulation in which other receptors are present can perhaps be explained on the supposition that it responds to the movements of the leg when the animal is not walking and when no complicated co-ordination of leg movements is needed. When, however, adjustments of the locomotory mechanism are required, a finer apparatus must come into action.

The superiority of this finer apparatus lies evidently (*a*) in the shifting of the cell bodies of all its neurons into the central nervous system, thus offering similar advantages as in the nervous system of vertebrates, and (*b*) in the considerable thickness of the afferent fibres facilitating the speedy transmission of the impulses in the reflexes co-ordinating the movements of the legs.[1] The abundance and variety of these fibres is indicative of the complexity of their functions and is further evidence of a far advanced evolutionary stage of the coxal receptors compared with those consisting of rows of sensory nerve cells. In the latter it is only the variations in size of these cells and in thickness of their axons which point to some differences, probably of graded character, of their function, whereas in the three receptors in the coxa there are several nerves and the role of each of them poses a problem which can only be solved by physiological experiments. Structural differences only occasionally afford some clue. One point concerning the thinner fibre *P* in the muscular receptor is worth mentioning. In crabs it can be seen that this fibre is confined to a small area of the same strand into which the thick fibre *S* penetrates (Text-fig. 5, *Carcinus b*), and as far as could be ascertained the end-filaments of the two fibres do not mix. Such a disposition would indicate that this fibre *P* is unlikely to have an inhibitory or excitatory character and that it probably responds to the variations of tension of the same strand as the fibre *S*, but transmits it at a lesser speed. In the levator receptor the thick and thin nerve fibres also appear to have independent areas of termination, but the pictures of their distribution are less distinct owing to the abundance of various nerve branches. Moreover, fibres which look much alike can certainly be physiologically different. Since, for instance, the thick fibres of the levator receptor, each of them much thicker than the strand on which they end, are at least two in number, it seems unlikely that they would have an identical function.

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[1] The situation of the cell bodies of the receptor neurons in the thoracic ganglia could not yet be ascertained. The direction of the course of the thick sensory fibres of the muscular receptor traced in *Homarus* leads to a group of smaller cells, but not the smallest ones, situated laterally to the largest cells in the postero-ventral region of the ganglion.

Thus, as far as the histological evidence goes, it can be stated that if experiments could show that a proprioceptor in the coxal region is capable of transmitting impulses to several neurons in the central nervous system, to excite some of them and inhibit others, and to do it at different speeds, there would be no reason to question whether it has enough nerve elements for all these various functions.

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SUMMARY

In the coxal region of *Eupagurus bernardus* the following receptor organs have been found: (1) a muscular receptor spanning the thoracico-coxal articulation, its innervation, in which several neurons take part, being arranged on a similar pattern as in *Carcinus*; (2) two innervated elastic strands running along the bundles of mm. levator and depressor basipoditis respectively and inserting into the tendons of these muscles; (3) a coxo-basipodite receptor consisting of connective tissue strand with numerous bipolar nerve cells ending on it.

With the exception of the coxo-basipodite receptor, all sensory neurons of these organs have their cell bodies located in the central nervous system.

It is suggested that these receptors convey impulses elicited by the movements of the legs and some hypotheses concerning the role of each of them are put forward.

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EXPLANATION OF PLATE I

All photographs were made from preparations of *Eupagurus bernhardus* stained with methylene blue, fixed in ammonium molybdate and mounted in xylol-dammar. Figs. 1, 3, 5 and 6 are made with the same magnification but the respective preparations were obtained from animals of various sizes.

Fig. 1. *MR*, thoracico-coxal receptor of the 2nd left peraeopod in connexion with the thoracic ganglion. The thin fibre crossing obliquely the nerve trunk in the left lower corner is the strand of the levator receptor (displaced).

Fig. 2. Proximal part of the thoracico-coxal receptor of the 2nd left peraeopod. *T*, fibre ending on the tendon.

Fig. 3. Thoracico-coxal receptor of the 2nd left peraeopod, showing its distal attachment.

Fig. 4. Thoracico-coxal receptor of the 5th left peraeopod with nerve fibres branching before reaching the muscle. *mot.*, motor nerve.

Fig. 5. Levator receptor of the 2nd left peraeopod with four nerve branches ending on the strand; cf. Text-fig. 3B.

Fig. 6. Depressor receptor of the 3rd peraeopod. This preparation was made from a much larger animal than that shown in fig. 5.

Fig. 7. Part of the levator receptor of the 3rd right peraeopod showing the nerve fibre at the point of meeting of the main and additional strands.

Fig. 8. Thick and thin fibre ending on the strand of the levator receptor. Same magnification as fig. 7.

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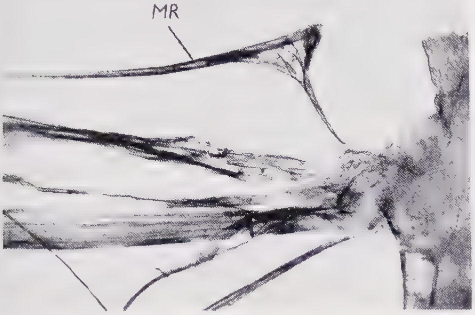

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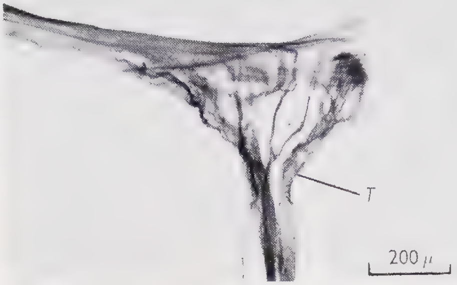

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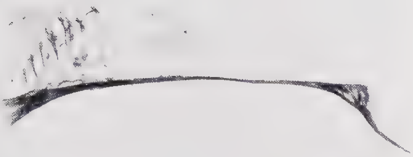

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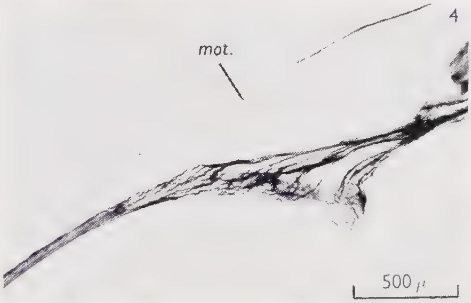

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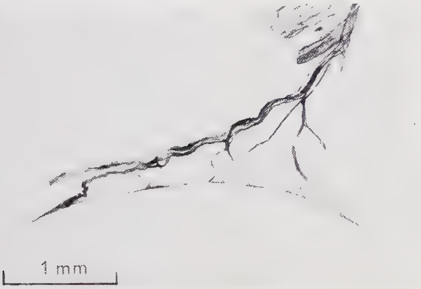

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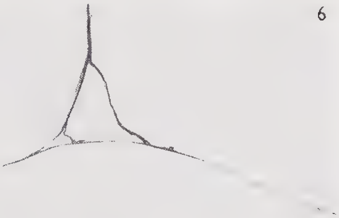

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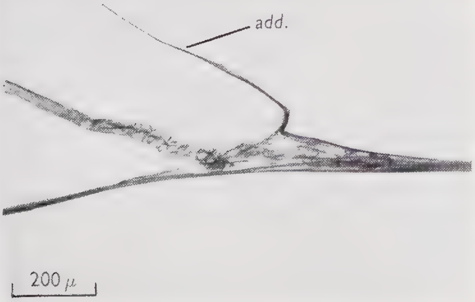

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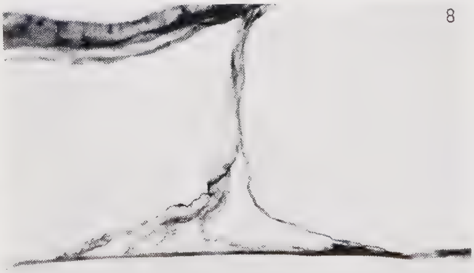

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STUDIES ON THE GROWTH OF MARINE PHYTOPLANKTON

I. *ASTERIONELLA JAPONICA* GRAN

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(Text-figs. 1–14)

Although the basic role of planktonic algae in the economy of the seas is well recognized, knowledge of their requirements for growth is still meagre. Besides work in which certain of these organisms are used as convenient objects for specialized physiological study there is need for more general investigations on the effects of different chemical and physical factors on the growth of species representative of the main groups of phytoplankton. Apart from their value for the understanding of the growth of natural populations, investigations of this latter type are essential for assessing the possibilities of the mass culture of planktonic algae for economic purposes. The object of the investigations to be described in this and subsequent papers was to obtain general information on the growth of single representatives of each of the three main classes of marine phytoplankton. The first of these to be considered is the diatom *Asterionella japonica* Cleve & Müller ex Gran.

Previous work on the growth of marine diatoms has been summarized by Harvey (1955), and most of the important references on their nutrition are given by Provasoli, McLaughlin & Droop (1957). Among studies particularly concerned with the growth rates of these organisms that of Braarud (1937) is noteworthy as having been the first to have been made with controlled temperature and illumination. In a subsequent paper Braarud (1945) has recorded the relative growth constants of many species grown in unialgal culture under such conditions. Recent studies on the effects of a factor of particular importance for diatom growth, viz. silica supply, have been made with marine and freshwater diatoms (Jørgensen, 1953, 1955, 1957; Lewin, 1957). The photometric technique for measuring growth has been used by Gross & Koczy (1946) and by Spencer (1954).

DEFINITIONS

In this work 'growth' has been taken primarily to mean increase in cell numbers. However, because it is more conveniently determined and leaves the culture intact, optical density rather than cell density of algal suspensions has usually been used as the direct measure of growth. Optical density depends on cell size, pigment content and other factors as well as on cell numbers, but a means of obtaining at least approximate values for relative growth constants from optical density determinations has been devised.

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The term 'optimum' as applied to nutrient concentrations and the level of physical factors has often been used loosely. Rodhe (1948) distinguished between a potential optimum level realized only under ideal conditions and an actual optimum depending on other factors. The latter can be found not only for the rate of growth but also for the final population. The two may not be identical under certain conditions, that is the range of concentration of a nutrient allowing the maximum growth rate may not extend to concentrations high enough for the attainment of maximum cell crop. As the magnitude of the latter is dependent on many factors it seems convenient to determine and express nutrient levels in terms of requirement per cell so that limitations by each factor can be readily calculated. The number of cells produced in response to the addition of a particular amount of a nutrient has been termed the 'cell crop'. This has been taken as the difference between the final cell concentration in the control cultures without the addition and that in cultures to which the nutrient was added.

METHODS

Glassware was cleaned by soaking in chromic acid followed by thirty complete rinses with tap water and three with distilled water. Steaming was found unnecessary. Pyrex glass culture vessels were used, either 50 ml. boiling tubes or 100 ml. conical flasks, plugged with non-absorbent cotton wool. They were maintained in a constant-temperature water bath, usually at 20° C, with underwater fluorescent lighting providing 5000–9000 lux.

The system used for aerating boiling-tube cultures was a modified form of that used by Spencer (1954). Compressed air from a diaphragm pump passed through the sintered-glass discs of three wash bottles of distilled water to a manifold of twelve needle valves. Through each valve it passed to a cotton-wool filter which was heated to prevent condensation (Fogg, Smith & Miller, in the press). This was attached by rubber tubing to the arm of a culture tube. This consisted of a normal boiling tube with a fine-bore glass tube sealed into the side, about 3 cm from the top, and passing down the inside against the wall to end in a jet at the bottom. This culture vessel was plugged with cotton wool and placed in the constant temperature tank.

The three media used for experiments on *Asterionella* are shown in Table 1. To avoid precipitation phosphate was added after autoclaving. Aseptic techniques were always employed. Media were sterilized by autoclaving at 15 lb. for 15 min. The media used for testing for contamination were those of Spencer (1952) made up with undiluted natural sea water.

When necessary, inocula were washed by aseptic centrifuging. The culture used was pipetted into a sterile centrifuge tube and a cotton-wool plug tied on. After centrifuging the supernatant was drawn off with a sterile pipette and replaced with sterile medium. After repeating the process, if necessary, the cell suspension was used as an inoculum.

TABLE 1. THE COMPOSITIONS OF THE MEDIA USED FOR *ASTERIONELLA*

	AK In sea water	AR_N In sea water	AR_A In distilled water
KNO_3	2·0 mM	2·0 mM	2·0 mM
K_2HPO_4	0·2 mM	0·2 mM	0·2 mM
$FeCl_3$	10 μM	5·5 μM	5·5 μM
$MnCl_2$	1·0 μM	—	—
Na_2SiO_3	20 μM	100 μM	100 μM
$ZnSO_4$	—	2·5 μM	2·5 μM
$MnSO_4$	—	20 μM	20 μM
$CoSO_4$	—	0·05 μM	0·05 μM
$CuSO_4$	—	0·02 μM	0·02 μM
H_3BO_4	—	550 μM	970 μM
NaCl	—	—	401·5 mM
$MgCl_2.6H_2O$	—	—	50·2 mM
Na_2SO_4	—	—	27·6 mM
$CaCl_2$	—	—	10·9 mM
KCl	—	—	8·91 mM
$NaHCO_3$	—	—	2·28 mM
KBr	—	—	0·806 mM
$SrCl_2.6H_2O$	—	—	0·150 mM
NaF	—	—	0·0714 mM
EDTA	—	0·342 mM	0·342 mM
Tris	—	8·25 mM	8·25 mM
Soil extract	20 ml./l.	—	

Growth was estimated by cell counts alone or in combination with optical density measurements. For cell counts a 1 ml. sample was removed from each culture and kept in a stoppered tube with solid iodine which killed and stained the cells and increased their specific gravity. The sample was shaken vigorously, which broke up the colonies to some extent, and the cells counted with a haemacytometer. Between 800 and 1000 cells were counted from each sample. Counts on twelve groups of 800 cells from the same sample gave a standard deviation of ± 6·0% of the mean. For optical density (OD) determinations a Unicam absorptiometer UIC no. 6117 was used. Culture tubes were selected to fit the compartment and marked so that they were always placed in the same way round. Appropriate blanks were used. Absorption was measured at the chlorophyll absorption peak, 680 mμ. The OD was found to be directly proportional to cell concentration up to 2500 cells/mm^3. The units used for OD were arbitrary.

For comparison between cultures it was necessary to express growth rates in terms of cell numbers. It was observed that the OD/cell varied according to the conditions under which the cells were grown and according to their ages and could therefore change during the course of an experiment. The initial cell concentration and also that at or near the end of the logarithmic growth period was determined by direct counting. The logarithmic rate of increase in OD was known, but if the OD/cell had changed this would not be equivalent to the logarithmic rate of increase in cell concentration. If it is assumed that the change in OD/cell occurs progressively during division an approximate way of calculating the relative growth constant k for cell numbers is to extrapolate the straight line on the graph of $\log_{10}$ OD to the levels of OD at the time the cell counts were made, and take the corresponding time as that needed to produce the observed change in cell concentration by exponential growth. This is shown diagrammatically in Fig. 1. The relative growth constant k is then given by the usual formula:

$$k=\frac{\log_e N_T-\log_e N_0}{t}=\frac{2\cdot 3}{t}(\log_{10} N_T-\log_{10} N_0).$$

Where N_T = the cell concentration at time T; N_0 = the cell concentration at time 0; and t = the time in days.

There was sometimes considerable variation of the relative growth constant from experiment to experiment due to variations in the age of the inoculum (p. 409). This did not affect the results within each experiment as controls were always included. Comparisons between separate experiments have been made only when the inocula were similar.

Cultures were invariably prepared in duplicate or triplicate, Student's t-test being used to test the statistical significance of the differences in growth observed in the experiments.

EXPERIMENTS

Isolation

Asterionella japonica was isolated into unialgal culture from a tow-net sample from the Hamble River, Hampshire, in October 1953.

Attempts to free this diatom of bacteria were unsuccessful. It failed to grow on a solid sea-water agar medium, and could thus not be separated from the bacteria by plating techniques. The use of antibiotics also proved unsatisfactory. The diatom was grown in concentration ranges of penicillin and streptomycin similar to those used by Spencer (1952) and also in a range of solutions of chloramphenicol. The cell concentrations after about 10 days' growth are shown in Fig. 2. Before testing for contamination the algae were subcultured to ensure sufficient dilution of the antibiotic for the development of any bacteria present. The results of the tests showed that certain colonies of bacteria were unaffected by concentrations of any of the antibiotics tolerated by the diatom. It is possible that further exposure or a mixture of the substances might have been effective. The curious increase in cell concentration with the rise of penicillin from 60 to 3000 μg/ml. will be discussed later. Finally, small colonies were washed by the method described by Pringsheim (1946). As it seemed possible that the diatom was dependent on bacteria for organic growth substances, the washed cells were inoculated into a variety of organic media which contained small quantities of soil extract, peptone, liver extract, amino acids and vitamins (including cobalamin). Growth of *Asterionella* took place only in medium AR without organic addition and then there was bacterial contamination. Most of the remaining media were contaminated. It seems likely that this was due to adherence of bacteria to the diatom frustules.

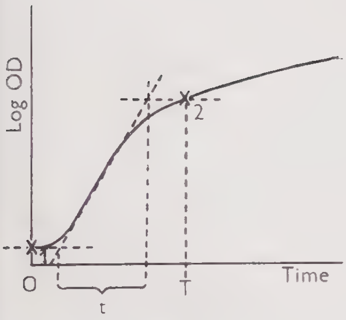

Fig. 1

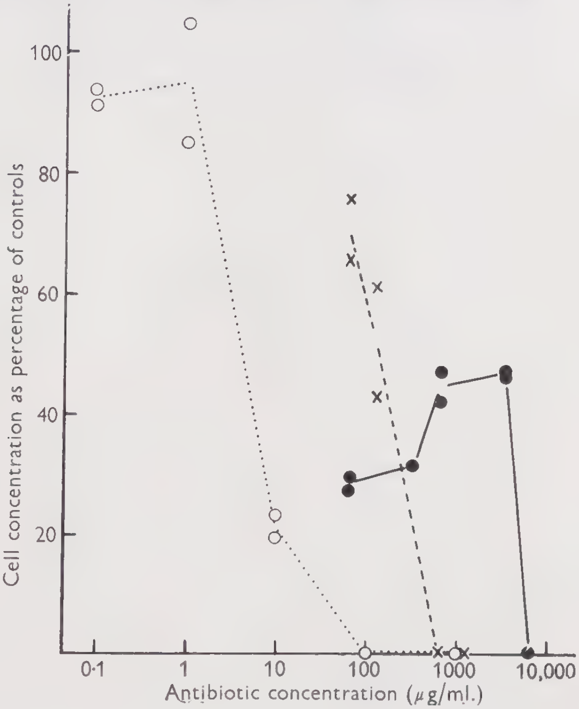

Fig. 2

Fig. 1. Diagram of the method of calculating the relative growth constant. The cell counts were made at times 0 and T, when the optical density readings were X_1 and X_2, respectively. The time interval t was used in the usual formula for the calculation of the relative growth constant k.

Fig. 2. Cell concentrations of *Asterionella* after 10 days' exposure to various antibiotic concentrations, expressed as percentages of those in controls without antibiotic additions. —●——●—, penicillin; –×–––×–, streptomycin; ··○···○··, chloramphenicol.

With the failure to obtain a bacteria-free culture of *Asterionella*, studies were made of the nutritional requirements of the alga in unialgal but not pure culture. Most of the studies were on mineral nutrition and the effects of physical factors, so that complications due to the presence of bacteria were probably minimal, and identical in all the cultures of a single experiment with equal inocula.

Growth

An example of the growth curve of *Asterionella* in stagnant medium in boiling tubes is shown in Fig. 3. There was a lag period of 0–20 h followed by an exponential phase with a relative growth constant usually of $k = 0{\cdot}7$–$1{\cdot}2$. This gave way, after less than 100 h from inoculation, to a more or less linear phase in which the inward diffusion of carbon dioxide was evidently limiting.

The pH of the culture medium normally rose during logarithmic growth from 8·0 at inoculation to about 8·5. During the phase of slower growth the pH remained more or less constant.

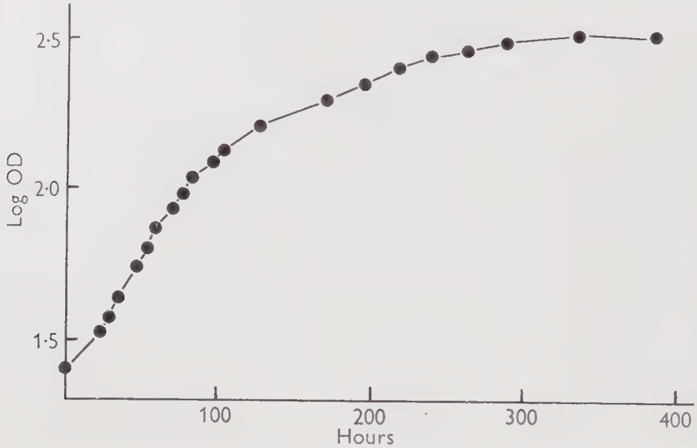

Fig. 3. The growth curve, determined by optical density readings, of a culture of *Asterionella*.

Nitrogen supply

Media were prepared containing added concentrations of potassium nitrate in artificial sea water of 0, 0·1, 0·3, 1·0, 3·0 and 10·0 mM. They were inoculated with cells that had been washed in nitrate-free artificial sea water. The cultures were aerated. The growth was followed by means of OD measurements and cell counts made when it had ceased.

Variation in nitrate concentration from 1 to 10 mM had no statistically significant effect on the relative growth constant. There was a marked difference in the OD/cell, however, that at 10 mM being three times that at 0·1 mM.

The cell crop is shown plotted against nitrate concentration in Fig. 4. The figure also shows the quantity of nitrate added to the medium divided by the crop obtained from it. This should be constant for nitrate-limited cultures. It is apparent that the two lowest concentrations were limiting. The mean quantity of nitrogen in these was 0·255 $\mu\mu$g atom N/cell. This can be regarded as the minimum required for these particular cells, since the method of

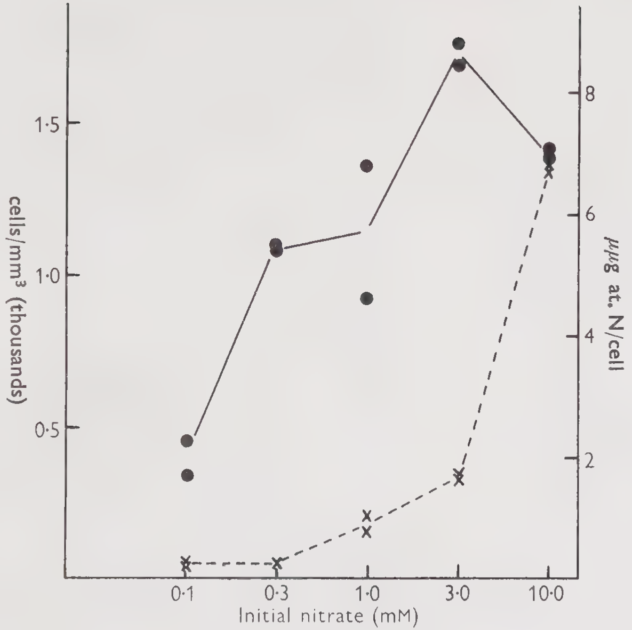

Fig. 4. The cell crop and nitrogen per cell of *Asterionella* in relation to concentration of potassium nitrate. —●——●—, cell crop; –×– – –×–, nitrogen/cell.

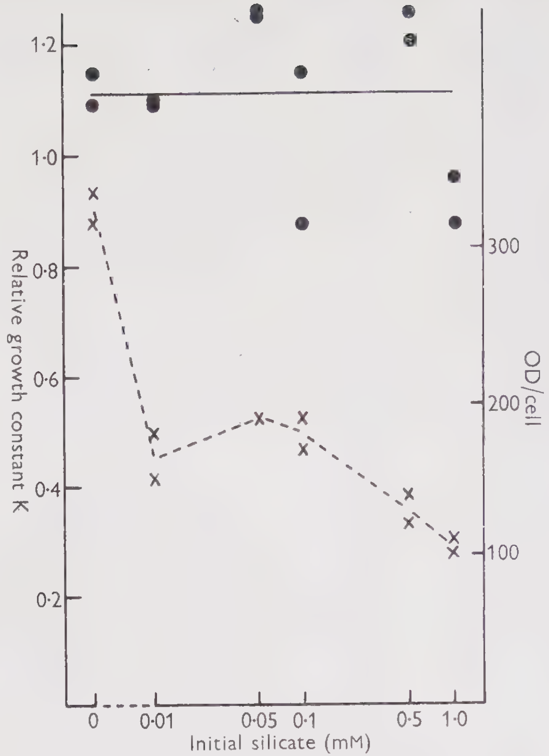

Fig. 5. The relative growth constant and final optical density per cell of *Asterionella* in relation to concentration of sodium silicate. —●——●—, *k*; –×– – –×–, OD/cell.

deducting the control cell concentrations should correct for error due to nitrogen added in the inoculum, or derived from nitrogen compounds in the air.

Phosphorus supply

The relative growth constant and OD/cell were not appreciably affected by variation in dipotassium hydrogen phosphate between 0·01 and 0·31 mM.

Silicon supply

The effect of different concentrations of silica on the growth of *Asterionella* was studied by using media with a range of 0 and 0·1–1·0 mM of added sodium silicate (Na_2SiO_3). Tris(hydroxymethyl)aminomethane, 'tris', (Provasoli *et al.* 1957) was used at a concentration of 8·25 mM to buffer the medium. The relative growth constants, determined from OD measurements only, showed no statistically significant variation (Fig. 5). The optical density per cell was markedly higher in the controls. This may have been because the diatom continued to produce pigment and other cell substances after cell division had been prevented by the depletion of the small quantity of silica from the glass and the inoculum.

The final cell concentrations in this experiment showed that the maximum population was reached with the addition of between 0·1 and 0·5 mM silicate. It was clear that, as noted by other workers, the thickness of the frustule varied with silica concentration and possibly the rate of growth, making it difficult to predict the cell crop that could be obtained from a given silica concentration.

Trace elements

The metal-chelator ethylenediamine tetra-acetic acid (EDTA) is now widely used in culture media for algae to prevent the precipitation of certain essential ions, particularly iron. It is necessary to accompany it by additional amounts of trace elements to compensate for those chelated. No experiments were made in the present investigation on the relative levels of ions necessary, but the proportions used by Provasoli *et al.* (1957) in the medium ASP 2 were tried in three concentrations together with several concentrations of EDTA. There was no growth in media with additions of trace elements giving 10 and 100 times the concentrations in ASP 2, in the presence of 0·342 mM of EDTA or less. The results of growth in various concentrations of EDTA with trace-element levels similar to those of ASP 2 are shown in Table 2. Concentrations of 0·0342–0·342 mM of EDTA seemed equally effective but more was inhibitory.

As the addition of this trace-element solution and 0·342 mM of EDTA proved successful it was considered a convenient way of providing minor elements and was used thereafter, in media AR_N and AR_A.

TABLE 2. GROWTH OF *ASTERIONELLA* IN MEDIA WITH VARIOUS EDTA ADDITIONS

	EDTA				
	0·0342 mM	0·171 mM	0·342 mM	1·71 mM	3·42 mM
	First experiment				
Cells/mm^3 at 485 h	2660	2110	3320	—	—
	2820	2400	1180	—	—
	Second experiment				
Cells/mm^3 at 340 h	—	—	1067	657	363
Relative growth constant *k*	—	—	0·967	0·801	0·773
	—	—	0·912	0·801	0·690

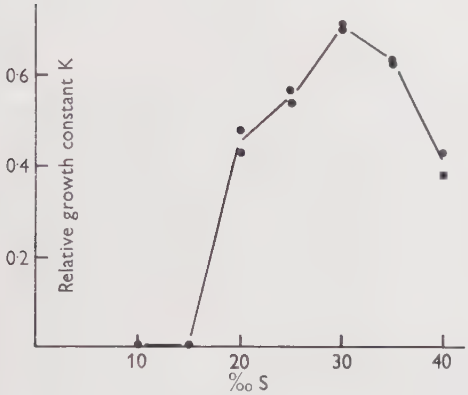

Fig. 6

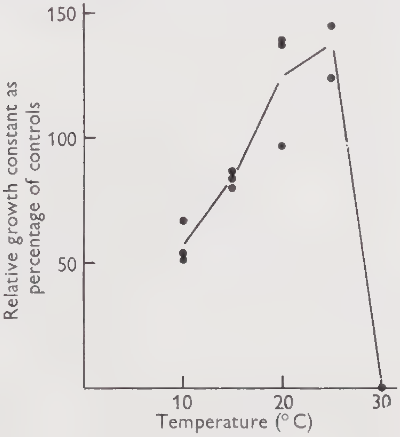

Fig. 7

Fig. 6. The relative growth constant of *Asterionella* in media based on sea water of various salinities.

Fig. 7. The relative growth constant of *Asterionella* at different temperatures (see text for further explanation).

Salinity

Asterionella was grown in natural sea water of various salinities produced by appropriate dilution or evaporation, with the usual nutrient additions. The relative growth constants, derived from cell counts only, are shown in Fig. 6. The optimum was between 30 and 35‰ S. The only salinities in which no growth took place were 10 and 15‰ S. It appears that *Asterionella* is fairly tolerant of salinities of 20 to 40‰ S or more. The relative growth constants at the extremes could possibly be increased by acclimatizing the cells to the different osmotic pressure.

Artificial sea water

Most of the experiments were made with natural sea water as a base. Although the principal constituents are remarkably constant there are several components of biological importance which may vary. It was therefore desirable to use artificial sea water if the medium was to be defined as closely as possible. A solution of the principal constituents found by Lyman and Fleming (Harvey, 1955, p. 137) in their analysis was used in place of natural sea water. When this, with the addition of nitrate, phosphate, silicate, EDTA and trace elements (i.e. medium AR_A) was compared as a medium with natural sea water with the same additions (i.e. medium AR_N), growth was identical. This result is equivocal, however, as the inoculum introduced 5 % of natural sea water. In a later experiment normal growth was obtained in artificial sea water after the inoculum had been washed in such a way that the original medium had been diluted to 0·02 %. Nevertheless, further subculturing indicated that a small quantity of natural sea water might be necessary.

Temperature

It was necessary for the experiments to be separated in time as the temperature could be varied only in one culture tank. A control tank enabled allowance to be made for differences in relative growth constants due to variation in factors other than temperature. Three cultures were placed in the control tank (always at 25° C) and three in the experimental tank at a different temperature in each experiment. Each relative growth rate of an experimental culture was expressed as a percentage of the mean of the controls. They are shown in Fig. 7, from which it appears that the optimum for the exponential phase was 20–25° C. In the later stages of growth of this culture of *Asterionella*, however, the bacteria seemed to overcome the algae more rapidly at 25 than 20° C so that the latter was a better working temperature. This may have been associated with progressive inhibition with time, as noted by Rodhe (1948). After 7 days at 30° C the diatom cells did not recover when returned to 20° C.

Light

The relative growth constant and optical density per cell was determined in various intensities of fluorescent ('white') light. Light intensity was measured with an Everett Edgcumbe autophotometer. The relative growth constants in three experiments under different conditions of temperature and aeration are shown in Fig. 8. The results were variable but it appears that there was saturation at 4000 lux and no inhibition at nearly 10,000 lux. The final OD/cell was almost unaffected by light intensity.

Aeration

As the length of the exponential growth phase of *Asterionella* seemed to be limited by the partial depletion of the carbon dioxide in the medium, an attempt was made to prolong the initial growth rate by artificial aeration. The result of this is illustrated in Fig. 9, in which the means of OD readings of aerated and stagnant cultures over the first 100 h are shown. Not only was the level to which exponential growth continued raised but the initial relative growth constant was increased. This latter could be due to keeping the cells in suspension or to the provision of carbon dioxide at a faster rate. These effects will be discussed in a later section.

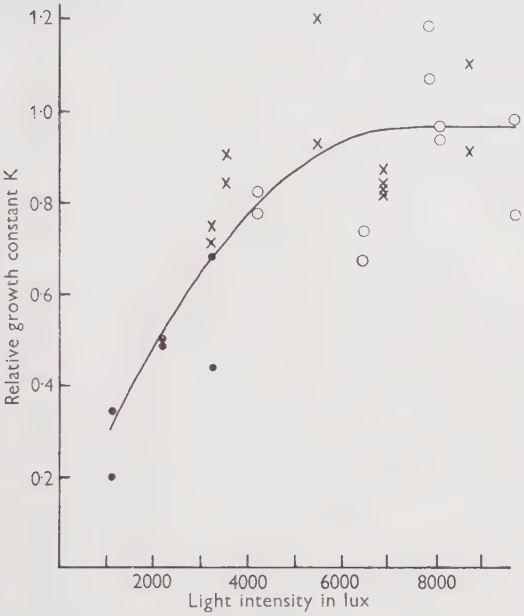

Fig. 8

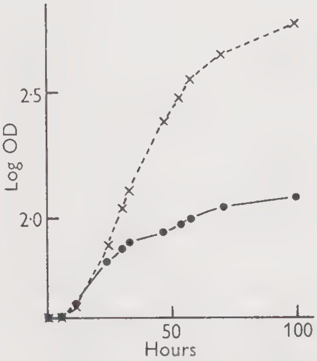

Fig. 9

Fig. 8. The relative growth constant of *Asterionella* grown under various conditions in different intensities of fluorescent light. ●, stagnant at 18° C; ×, aerated at 18° C; ○, aerated at 25° C.

Fig. 9. The growth curves, determined from optical density readings, of *Asterionella* in stagnant and aerated culture. —●——●—, stagnant; –×–––×–, aerated.

Hydrogen-ion concentration

The apparent importance of the effect of pH on the growth of *Asterionella* necessitated a more detailed study of this factor. In the first place a method was devised to control the pH of the medium during the growth of the diatom. Two series of vessels were prepared, an 'experimental' and a 'dummy' series. Each of these consisted of duplicate cultures at six different pH's. The two series were treated in the same way except that no attempt was made to keep the dummies sterile. After inoculation and at intervals during growth the dummy tubes were opened and the pH of each medium adjusted. The quantity of acid or alkali needed for this adjustment was then added aseptically in a sterile form to the experimental series. Growth determinations were made on the latter.

In the first experiment of this kind no buffer was included in the sea-water medium. It was found that the removal of carbon dioxide altered the adjusted pH so rapidly that it was impossible to control it by this method. The buffer tris was therefore added to the medium at a concentration of 41·2 mM. The relative growth constants at the different adjusted pH's are shown in Fig. 10. It is apparent that pH's from 7·5 to 8·25 were optimal but there was inhibition at 8·5 and 8·75.

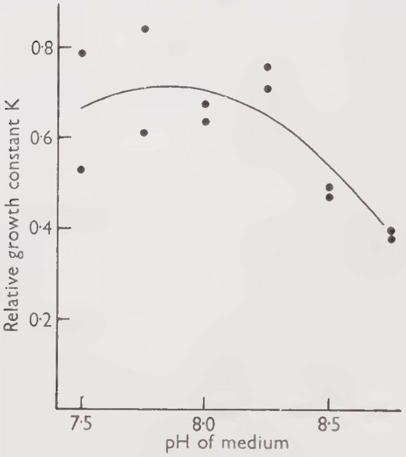

Fig. 10

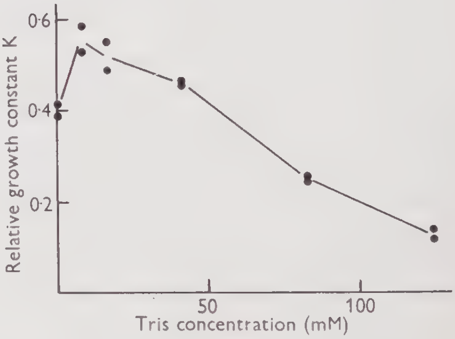

Fig. 11

Fig. 10. The relative growth constant of *Asterionella* in media adjusted to various pH's.

Fig. 11. The relative growth constant of *Asterionella* in relation to concentration of the buffer tris.

TABLE 3. GROWTH OF *ASTERIONELLA* IN MEDIA WITH AND WITHOUT TRIS

(Relative growth constant *k* (in triplicate).)

Without tris	0·380	0·437	0·366
With 8·25 mM tris	0·658	0·564	0·564

In using this buffer it was considered desirable to find the highest concentration that the diatom would tolerate. The relative growth constants were therefore determined in media with various additions of tris. These are shown in Fig. 11. There was stimulation at 8–16 mM, followed by inhibition with increasing concentration. Tris of greater purity caused a similar stimulation, as is shown in Table 3, the difference being statistically significant at the 1 % level. The possibility that tris stimulated growth through its buffering action seemed the most likely and was studied further.

The effect of the growth of *Asterionella* on the pH of stagnant medium has already been mentioned. In order to follow the rise under different conditions the growth of two cultures was studied in detail in each case, while further cultures under the same conditions were used successively for pH determinations. In this way the change in the pH of the medium was followed in the presence and absence of 8·25 mM of tris in stagnant culture and also in the absence of the buffer in aerated culture. The pH rose steeply during exponential growth in stagnant culture, but remained constant once this phase had ended. In the absence of tris it rose from pH 8·0 to 8·55 but in its presence only to 8·35–8·40. In aerated culture although the growth was much greater there was a negligible rise in pH. Thus, although the buffer was partly effective in preventing the pH change, it was not as effective as aeration.

TABLE 4. GROWTH OF *ASTERIONELLA* IN STAGNANT AND AERATED CULTURE WITH AND WITHOUT TRIS

(Relative growth constant *k* (in triplicate).)

	Stagnant			Aerated		
Without tris	0·695	0·619	0·663	1·18	1·16	1·17
		↕			↕	
With 8·25 mM tris	0·906	1·16	0·983	1·06	1·05	1·02

Arrows indicate a statistically significant difference at the 1% level.

If tris were stimulating growth solely because of its buffering action its effect would be masked under aerated conditions where little rise in pH took place in any case. An experiment was therefore made comparing the relative growth constants in media with and without tris, in stagnant and aerated culture. The results are shown in Table 4. It is apparent that instead of stimulating growth when the culture was aerated tris inhibited it, though only to the rate of stimulated stagnant growth. Aeration and tris thus had a similar action in accelerating growth. As in stagnant culture the cells settled to the bottom of the vessel, it would seem likely that this action was to prevent the pH in the vicinity of the cells from becoming inhibitory.

Unidentified inhibitory factor

The variation between relative growth constants from experiment to experiment was observed to be large, and it was therefore important always to have controls. In order to investigate the possibility that this variation might be associated with the size or age of the inoculum, identical culture media were inoculated with different amounts of inoculum from cultures of three different ages. The relative growth constants were calculated in the usual way and are shown plotted against the size of the inoculum in Fig. 12. There was evidence of a decrease in the constant both with increase in age and in increase of size of the inoculum. The extent of the variation with age was more than sufficient to account for the differences between the relative growth constants in different experiments. The decrease with increasing age may have been due to an increasing proportion of non-viable cells in the inoculum. The effect of size cannot be explained in this way. The relative growth constant should be the same for a given proportion of viable cells with any inoculated number of these. A possible explanation might be that some inhibitory substance was carried over in the medium with the inoculum.

In an earlier experiment cultures were prepared with only half freshly autoclaved medium. The other half was Seitz-filtered sea water in three of the cultures and Seitz-filtered old *Asterionella* culture medium in another three. The relative growth constants (from OD measurements only) are given in Table 5 and were significantly lower at the 2·5 % level in the cultures with the old medium. This seemed to indicate the presence of an inhibitory substance in the old culture.

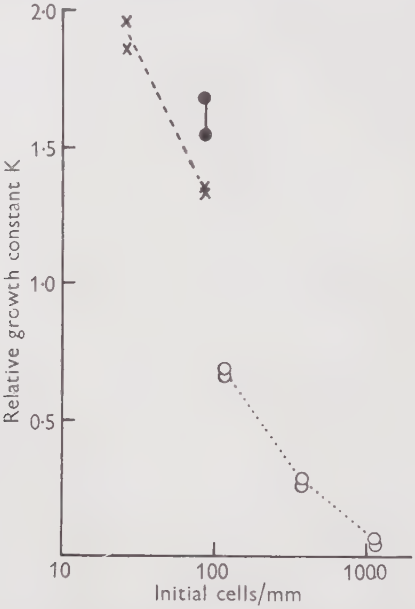

Fig. 12. The relative growth constant of *Asterionella* in cultures inoculated with different amounts of inoculum from cultures of three different ages. —●——●—, inoculum 37 h old; –×–––×–, 190 h old; ··○····○··, 890 h old.

TABLE 5. GROWTH OF *ASTERIONELLA* IN PARTLY SEITZ-FILTERED MEDIUM

(Relative growth constant *k* (in triplicate).)

Half medium of sea water	0·442	0·529	0·465
Half medium of aged culture medium	0·331	0·412	0·343

Further experiments on this factor were inconclusive. It is possible that if an inhibitor was produced at all, it was produced only under certain conditions.

Unidentified stimulatory factors

It has already been suggested that *Asterionella* may require organic substances for growth and that it normally obtains these from bacteria in crude culture.

In the experiment on the range of penicillin tolerated by the diatom it was found that there was a stimulation of growth with increasing concentration of antibiotic within the range of 60–3000 μg/ml., although in general penicillin was inhibitory (Fig. 2, p. 401). It is likely that the inhibition at 60 μg/ml. was due to the effect of the antibiotic on the bacteria, inactivating them and thus slowing down the production of a substance necessary for diatom growth. Increasing quantities of penicillin caused stimulation either by providing the required substance or substances as chemical impurities, or by releasing them into solution from the bacterial cells as a result of autolysis. This might be taken as an indication that one or more organic substances stimulated the growth of *Asterionella*.

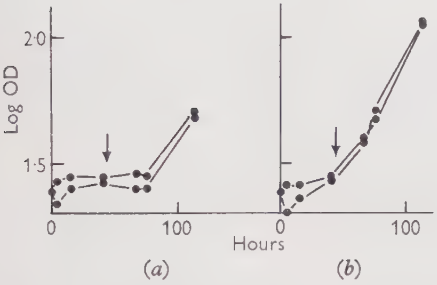

Fig. 13

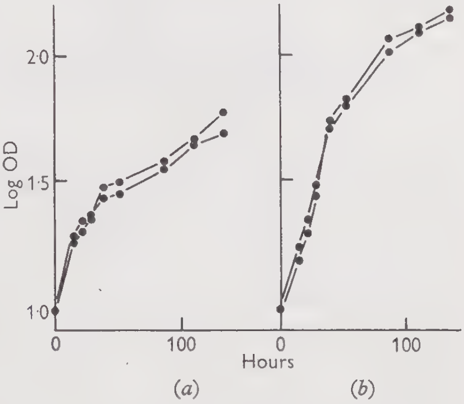

Fig. 14

Fig. 13. The growth curves of *Asterionella* in a synthetic medium, (*a*) without the addition of old medium, and (*b*) with the addition of 1 ml. of the supernatant of an aged culture. The arrow indicates the addition to each of 1 ml. of natural sea water.

Fig. 14. The growth curves of *Asterionella* in a natural sea water medium, (*a*) without addition, and (*b*) with the addition of 1 ml. of the supernatant of an aged culture.

Further evidence for this came from an experiment in which the inoculum was washed in artificial sea water for two successive subcultures in synthetic medium. The washing involved aseptic centrifuging and many of the bacteria would have remained in the supernatant and been discarded. Thus the proportion of bacterial to algal cells would have been reduced. Four of these cultures were grown, two as controls and two with the addition of 1 ml. of the supernatant of an aged medium. The growth curves are shown in Fig. 13. Growth was clearly stimulated by the presence of the aged medium, but only after the addition of 1 ml. of natural sea water to each culture. This may have been coincidental, though a lag of that length had not previously been observed.

In the next experiment the medium was based on natural sea water and an addition made from the same aged medium. The growth curves are shown in Fig. 14. Growth was immediate in all the cultures, but considerably better in those with the aged medium addition than in the controls. The absence of lag suggested that some substance, possibly cobalamin, present in natural sea water had to be present before growth could take place in the previous experiment. But it is clear that some further substance was derived from the aged medium. In a further experiment an addition of the same aged medium was made, in both autoclaved and unsterilized forms. The unsterilized addition was clearly more effective than the sterilized. Thus, some thermolabile factor is stimulatory to the diatom, but it cannot be said whether this is produced by *Asterionella* itself or by bacteria.

These results will be discussed together with those for other species on a later occasion.

This work has been carried out under extra-mural contract with the Institute of Seaweed Research. We are grateful to the Institute for a maintenance grant made to one of us (J. M. K.) and to its Director and Staff for their interest and help.

SUMMARY

A planktonic marine diatom, *Asterionella japonica*, has been grown in unialgal, but not bacteria-free, culture under controlled conditions and its growth has been measured by means of optical density determinations and cell counts in combination.

The relative growth constant has been found to be usually between 0·7 and 1·2 $\log_e$ units per day, and to be little affected by variation of the concentration of nitrate, phosphate and silicate within wide limits. Rapid growth occurred in waters having salinities between 20 and 40 ‰ S. The optimum temperature for growth was 20–25° C and the optimum light intensity from 4000 to 10,000 lux. The relative growth rate was affected most markedly by variation in hydrogen-ion concentration. This was manifest in stagnant cultures in which the rise of pH above 8·3, caused by absorption of carbon dioxide, resulted in the inhibition of growth. This could be countered by increased aeration or by the addition of the buffer tris(hydroxymethyl)aminomethane.

Evidence has been obtained which suggests that *A. japonica* requires a thermostable substance present in natural sea water and that a thermolabile substance stimulating its growth is produced in culture.

Final yields of the order of 4000 cells per mm^3 were obtained and, within limits, were dependent on the amounts of nitrate and silicate supplied. The nitrogen requirements per cell was determined as 0·255 $\mu\mu$g atom. Indications were obtained of the presence of a growth-inhibiting substance in the filtrates from old cultures.

The optical density of the cells was increased at high nitrate concentrations and when exhaustion of silicate prevented cell division.

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THE IMPORTANCE OF BACTERIA IN LABORATORY EXPERIMENTS ON REARING THE LARVAE OF *OSTREA EDULIS* (L.)

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Observations made by the writer during the summer of 1955 suggested that the density of the bacterial flora which develops when oyster larvae are reared in small glass vessels is inversely correlated with the number of spat settling (Walne, 1956). From the nature of the observations it could not be determined whether the bacteria were affecting the development of the larvae or whether the properties of the sea water which resulted in a dense bacterial population were unfavourable to larval growth. This was clearly a matter which required further investigation. Investigators rearing marine larvae have commonly found that when a standard rearing technique is employed over a period, considerable differences occur in the proportion of different broods of larvae which grow to metamorphosis. There has been no satisfactory explanation for this phenomenon, although it has been assumed to be due to the presence or absence of minute quantities of various organic compounds in the water (Wilson, 1951; Loosanoff, 1954). The observations recorded in this paper suggest that a partial explanation could be that batches of sea water differ in the number of bacteria which they will support when confined in laboratory vessels, and these bacteria in turn affect the growth of the larvae.

EXPERIMENTAL METHOD

To test the hypothesis, it is necessary to rear larvae in an environment where the bacterial density is low, and to compare the number of larvae which settle as spat with that in control experiments where the normal bacterial population develops. Sea water can be freed of bacteria by various methods. Filtration is perhaps the most satisfactory as the chemical composition of the water is less likely to be altered than by methods which employ heat, but Wilson & Armstrong (1954) have shown that it is very difficult to collect large samples of bacteria-free water by this method. However, although experiments could be set up with bacteria-free water, the larvae could not be freed from bacteria. At the rearing temperature (20° C) marine bacteria divide frequently, and even if only a few are present initially a dense population soon develops. It was therefore decided to use antibiotics, since these would either partially or completely control the bacterial population, without removing any substance from the sea water which might be required by the larvae. Oppenheimer (1955) has used antibiotics to good effect in hatching marine fish eggs. Some experiments in which lamellibranch larvae were grown for a time in the presence of antibiotics have been reported by Davis & Chanely (1956).

A series of experiments was made in 1956 in which the success of cultures of larvae grown under standard conditions was tested against identical experiments to which penicillin G had been added. The results were promising and a more searching series of experiments, which also included the use of streptomycin and chloromycetin, was made in 1957.

Broods of larvae were obtained from oysters kept in running sea water heated to 20–22° C and enriched with cultures of *Phaeodactylum*. The larvae were liberated naturally and were removed by filtering the water through bolting-silk. After washing in filtered sea water, the larval density was determined by counting samples of an even suspension of larvae. The appropriate volume of larval suspension was then dispensed into hard glass beakers, each containing 1 l. of sea water which had previously been passed through filter-candles to remove silt and the plankton, with the exception of a few bacteria. The average number of larvae added was 1300 per l. The larvae were fed on the flagellate *Isochrysis galbana* Parke, which had been cultured in standard 'Erdschreiber' medium (Gross, 1937). Sufficient was added to give an initial density of about 50 cells per mm^3. Further culture was added daily to keep the food density at about this figure.

In 1956 all experiments of a series stood together in the same water-bath, the temperature of which varied between 20 and 23° C. In 1957 the beakers stood on a bench in a laboratory, the controlled temperature of which varied between 21 and 22° C. All were gently stirred by air bubbling from a glass jet held near the bottom of the beaker. When eyed larvae were observed, spat collectors, in the form of clean mussel shells which had been strung on stainless-steel wire, were suspended in the beaker. When the spatfall was complete, generally 20–30 days after the beginning of the experiment, the total settlement on the mussel shells and the sides and bottom of the beaker was carefully counted. Samples of the larvae which had failed to metamorphose were measured.

The bacterial flora was studied at intervals by plating either 1·0 or 0·1 ml. samples on ZoBell's medium No. 2216 (ZoBell, 1946), and incubated at 22° C for 48 h. When the flora was dense, ten microscope fields were counted on each plate. The size of the field was adjusted where possible so as to count ten to twenty colonies per field. At very low densities (<1000 per ml.) the colonies were counted directly on the whole plate. The low density populations (0–20,000 per ml.) have thus been estimated fairly accurately; the higher densities, because of the crowding of the colonies on the plates, less precisely.

RESULTS

In 1956 ten series of experiments were made, each series using a different brood of larvae. In each series two treatments were tested. A control treatment, set up with filtered sea water, larvae and food, was compared with a second treatment which was identical, except that sufficient of the sodium salt of penicillin G was added at the commencement to give a concentration of 50 i.u. per ml. In eight of the ten series of experiments made, the two treatments were duplicated. In 1957 a further five series of experiments were made. In the first two series the treatments were triplicated and in the other three quadruplicated. The concentration of penicillin G was selected by reference to the work of Cviic (1953) who showed that which would control many species of marine bacteria. Spencer (1952) had already shown that it was relatively harmless to *Isochrysis* at this concentration.

There was, as expected, a marked difference in the density of the bacterial flora between penicillin experiments and the controls (Table 1). In 1956 the bacterial flora was estimated at the end of the first 24 h and every other day thereafter. In the controls the mean density was about 19,000 per ml. after 24 h, but in the penicillin experiments the density was less than 1000 per ml. On the third day the mean density was 48,000 per ml. in the penicillin experiments, whereas in the controls it had declined slightly to about 16,000. This increase of the bacteria in the penicillin experiments may have been due either to the penicillin breaking down and losing its bacteriostatic properties, or to the development of penicillin-resistant bacteria in the experiments. By the fifth day the numbers in the two treatments were approximately equal.

In the 1957 experiments bacterial estimations were made when the experiments were first set up, but before the addition of any antibiotic, and then every other day (except for series 4 where the initial count was omitted). The water used generally contained a fairly high bacterial population, probably acquired in the day or so which it took to pass through the filtering unit (water was generally used the day after filtering). The average initial bacterial population was 24,000 per ml. in series 1, 2, 5; in series 3 it was abnormally high (230,000). The average of all five series of experiments on the second day was 75,000 in the control, and < 1000 in the penicillin experiments. Thereafter, the density of bacteria declined rapidly in the controls. In the penicillin experiments, the numbers rose a little but not so markedly as in 1956. A general picture of the changes in bacterial density in this type of experiment was given by series 5, 1957 (Table 1), where the bacterial flora was observed daily for the first 6 days. The initial population was fairly high (45,000). In the controls it rose to 88,000 on the first day, and thereafter declined by about half each day, reaching an average of about 2000 per ml. on the sixth day. The penicillin was added after 24 h; the effect was to reduce the bacterial population, which had had time to build up, to only about half that found in the controls.

Experiments were made in 1956 to see whether alterations in the dosage of the penicillin would control this outburst of bacteria. No success was obtained and no improvement in the growth and metamorphosis of the larvae was observed; in many cases the reverse was the case. Apparently many of the bacteria which developed were penicillin-resistant species or strains. Two types of experiments were tried. In one 50 units per ml. were added at the beginning, and a further 50 units every third day. In the second type of experiment 100 units were added at the beginning and a further 50 units every third day. In many experiments of both series very dense bacterial populations gradually developed.

TABLE 1. SUMMARY OF THE BACTERIAL POPULATION IN THE EXPERIMENTS OUTLINED IN TABLES 3 AND 4

(The bacteria are recorded to the nearest thousand per ml. The concentrations of antibiotic used are shown in Tables 3 and 4.)

	Day ...	0	1	2	3	4	5	6	7
				1956					
Series 1	Control	—	240	—	5	—	43	—	46
	Penicillin	—	2	—	26	—	39	—	47
2	Control	—	—	60	—	8	—	56	—
	Penicillin	—	—	1	—	24	—	135	—
3	Control	—	84	—	—	23	—	11	—
	Penicillin	—	23	—	—	127	—	19	—
4	Control	—	15	—	14	—	8	—	13
	Penicillin	—	3	—	57	—	3	—	10
5	Control	—	7	—	6	—	5	—	5
	Penicillin	—	1	—	57	—	7	—	24
6	Control	—	7	—	12	—	7	—	—
	Penicillin	—	0	—	95	—	37	—	—
7	Control	—	2	—	24	—	11	—	—
	Penicillin	—	1	—	46	—	129	—	—
8	Control	—	1	—	18	—	16	—	—
	Penicillin	—	0	—	43	—	8	—	—
9	Control	—	—	—	49	—	4	—	9
	Penicillin	—	—	—	51	—	4	—	46
10	Control	—	3	—	6	—	11	—	—
	Penicillin	—	1	—	15	—	30	—	—
				1957					
Series 1	Control	5	—	118	—	6	—	6	—
	Penicillin	5	—	<1	—	1	—	1	—
2	Control	23	—	115	—	6	—	<1	—
	Penicillin	23	—	<1	—	1	—	2	—
3	Control	231	—	77	—	9	—	117	—
	Penicillin	231	—	<1	—	4	—	<1	—
	Penicillin and streptomycin	231	—	0	—	0	—	0	—
4	Control	—	—	11	—	<1	—	4	—
	Penicillin	—	—	3	—	<1	—	8	—
	Penicillin and streptomycin	—	—	0	—	0	—	0	—
5	Control	45	88	55	20	9	5	2	—
	Penicillin	45	61	23	5	7	4	3	—
	Penicillin and streptomycin	45	18	0	0	0	0	0	—

From these experiments three types of data about the larvae are available—the proportion of the larvae which metamorphosed and settled as spat, the maximum rate of growth of the larvae, and the size to which those larvae which failed to metamorphose grew before dying. These three sets of data will be considered in turn.

The yield of spat from an experiment is of primary interest, since settlement indicates that the larvae have completed their development in the conditions offered. In all, the settlement of fifteen broods of larvae has been compared in control experiments with those to which penicillin had been added. The mean number of spat per litre obtained with each of these fifteen broods with the two treatments is compared in Table 2. It will be seen that, on the average, more spat was always recorded in those experiments to which penicillin had been added and the significance of this difference has been calculated. Because the differences are log-normally distributed, it is necessary to transform the data into logarithms. A '*t*' test on the transformed data shows that these differences are significant at the $P = > 0\cdot001$ level.

TABLE 2. MEAN NUMBERS OF SPAT OBTAINED IN EXPERIMENTS WITH PENICILLIN

(The experimental medium contained 50 i.u. of the sodium salt of penicillin G which the otherwise identical control lacked.)

	1956										1957				
Series ...	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5
Control	258	222	192	0	107	135	218	0	0	0	89	19	107	203	0
Penicillin	300	320	290	48	330	140	411	8	42	0	225	715	573	1155	45
Excess spat in penicillin experiment	42	98	98	48	223	5	193	8	42	0	136	604	466	952	45

Larvae were measured from time to time and their sizes noted. The mean size of the larvae in the different experiments cannot be calculated as it was not practicable to measure sufficiently large samples. The largest size present was, however, probably accurately estimated. There was no tendency for the larvae to be larger in the penicillin experiments than in the controls on the same day. This conclusion applies, of course, only to those controls which were not unduly affected by bacteria. A good example is given by the experiments in series 4, 1957 (Table 4). The mean sizes of about forty larvae measured on the fifth and eighth days are shown below:

Day ...	0	5	8
Control (μ)	183	217	235
Penicillin (μ)	183	217	241
Penicillin/streptomycin (μ)	183	218	243

Growth was slow in this series but the yield of spat from those treated with antibiotics was very good (Table 3). If the bacterial population becomes very dense, then the larvae will eventually stop growing.

An examination of the sizes (Tables 3 and 4) to which those larvae which failed to metamorphose grew before dying shows that in many cases the whole larval population was affected by the addition of penicillin. For conciseness, the sizes at which larvae died have been aggregated into four groups corresponding to the principal stages in larval development:

Group	μ	
1	160–200	Size at liberation, little or no growth
2	210–250	Substantial growth, D-shape of freshly liberated larvae lost
3	260–300	Size at which the anatomical changes which presage metamorphosis take place, including development of eye spots. Many larvae settle at this size
4	> 300	Mature larvae. Most larvae settle before reaching this size

The significance of the differences in the proportions of larvae which grew into each size-group can be tested for each series of experiments by reference to the fourfold contingency tables published by Mainland, Herrera & Sutcliffe (1956). In one series (series 10, 1956), no larva grew out of the first size-group; these larvae were probably abnormal and can be disregarded. The difference in the proportion of larvae which grew into the 210–250 μ group between the penicillin experiments and the controls is significant ($P=0{\cdot}01$) in six of the eleven series of experiments with which we are concerned (series 4, 8 and 9, 1956, and series 1, 2, and 5, 1957). In all cases where a difference occurred, the larvae were larger when treated with penicillin. In the next size-group the differences are again significant ($P=0{\cdot}01$) in six of the eleven series of experiments (series 1, 4, 7, 8 and 9, 1956, and series 2, 1957). In one case (series 7, 1956), the larvae were larger in the control, but here many more larvae settled as spat in the penicillin experiments which probably reduced the proportion of larvae dying in the larger size-groups. The numbers in the fourth group, 300 μ, were generally small and considerably influenced by the number settling as spat.

As the larvae in the penicillin experiments were not observed to grow more rapidly than in the controls, the larger size at death of the larvae in many of the penicillin experiments must have been due to the larvae living longer.

In 1957 some experiments were made using streptomycin and chloromycetin. Cviic (1953) has shown that a mixture of penicillin and streptomycin is more effective for controlling marine bacteria than either antibiotic on its own. In series 1 and 2 (Table 4) a mixture of 50 units of penicillin G and 0·25 mg streptomycin sulphate per ml. was tried. This completely suppressed the bacteria for over 9 days but the effect on the larvae was variable. In the first series none of the larvae in the three replicates grew to more than 250 μ and they speedily died, which suggested that the streptomycin was harmful. In the second series, the larvae were killed by mistake in one experiment when they had grown to 280 μ. In the replicate there were 395 spat. The larvae, however, behaved abnormally in the early stages of the experiment. Their ciliary activity was extremely rapid and they did not close up as rapidly as

TABLE 3. SUMMARY OF THE RESULTS OF TEN SERIES OF EXPERIMENTS (1956) TESTING THE EFFECT OF ADDING 50 I.U. OF Na PENICILLIN G TO CULTURES OF OYSTER LARVAE

(C = control experiments. P = experiments to which penicillin was added.)

Series	Origin of parent oyster	Date started	No. larvae per litre	Type of expts.	No. of expts.	% of larvae which achieved each size group (μ)			No. of spat
						210–250	260–300	>300	
1	Isle of Lewis	19. iii. 56	1500	C	2	89	65	25	175, 340
				P	2	95	91	44	556, 43
2	1954, Brittany	4. iv. 56	1300	C	1	—	—	—	222
				P	1	—	—	—	320
3	Isle of Lewis	9. iv. 56	1600	C	1	—	—	—	192
				P	1	—	—	—	290
4	Brittany	3. vii. 56	1200	C	2	84	35	1	0, 0
				P	2	100	89	6	50, 45
5	1954, Brittany	3. vii. 56	1400	C	2	98	74	0	125, 89
				P	2	98	81	21	355, 305
6	River Colne	7. vii. 56	1500	C	2	93	73	16	75, 195
				P	2	91	58	12	230, 50
7	River Colne	7. vii. 56	1700	C	2	93	70	2	365, 70
				P	2	85	40	0	425, 397
8	1954, Brittany	7. vii. 56	1050	C	2	79	4	0	0, 0
				P	2	100	75	14	5, 10
9	River Colne	12. vii. 56	1300	C	2	80	12	0	0, 0
				P	2	96	32	0	8, 75
10	Brittany	5. viii. 56	1400	C	2	0	0	0	0, 0
				P	2	0	0	0	0, 0

TABLE 4. SUMMARY OF THE RESULTS OF THE 1957 SERIES OF EXPERIMENTS

(The parent stock was always from the river Colne. In series 5 the penicillin was not added until the experiment had been running for 24 h.)

			Antibiotics added per ml.				% larvae which achieved each size group (μ)			
Series	Date started	No. larvae per litre	Penicillin G (i.u.)	Streptomycin (mg)	Chloromycetin (mg)	No. of expts.	210–250	250–300	>300	No. of spat
1	27. iii. 57	1100	0	0	0	3	86	26	—	111, 73, 82
			50	0	0	3	94	42	—	292, 61, 323
			50	0·25	0	3	28	—	—	0, 0, 0
2	29. iii. 57	1300	0	0	0	3	78	8	—	0, 0, 58
			50	0	0	3	93	45	—	809, 798, 538
			50	0·25	0	2	69	8	—	0,* 395
3	1. iv. 57	960	0	0	0	4	68	12	—	230, 2, 197, 0
			50	0	0	4	Not recorded			612, 629, 332, 720
			50	0·05	0	4	Not recorded			545, 282, 387, 394
4	3. iv. 57	1400	0	0	0	4	96	51	—	749, 14, 29, 22
			50	0	0	4	91	58	—	1091, 1211, 1137, 1182
			50	0·05	0	4	Not recorded			1259, 992, 1292, 1082
5	7. v. 57	1400	0	0	0	4	65	1	—	0, 0, 0, 0
			50	0	0	4	97	9	—	0, 0, 0, 179
			50	0·05	0	4	95	31	—	752, 183, 404, 74
			0	0·05	0	4	51	—	—	0, 0, 0, 0
			0	0	0·025	4	98	28	—	0, 0, 0, 0

* This experiment was terminated early by accident.

usual. This behaviour seemed to be characteristic of experiments to which streptomycin had been added. In the next three series of experiments a mixture of 50 units penicillin G and 0·05 mg streptomycin sulphate per ml. was tried. Once again the bacteria were completely suppressed for at least 9 days. In two of the three series of experiments (3 and 4) the larvae did well, much better than the controls, but not significantly different from those to which only penicillin G had been added. In series 5 the result from the penicillin/streptomycin mixture was much better than in the control, or in that to which penicillin alone had been added, both in respect of the number of spat produced and in the size reached by the larvae before death. It seems probable that when the bacterial flora is very vigorous, or perhaps when certain species are present, then the penicillin/streptomycin mixture will give better results than penicillin alone.

One experiment was tried using chloromycetin; this is a wide spectrum antibiotic. The larvae grew well until there was an outburst of bacteria apparently resistant to this antibiotic, when they died. Chloromycetin may not, therefore, be as useful as the penicillin/streptomycin mixture and, as it costs at least two-thirds as much again, it may not have a place in large-scale rearing of larvae.

DISCUSSION

The proportion of larvae which develops to metamorphosis differs, under standard conditions, in different broods. An example of this is shown by the results of series 6, 7 and 8, 1956. These experiments were all started on the same day with the same batch of water, but the three broods differed considerably in the proportion of larvae which metamorphosed. One of the factors affecting their survival is described in this paper, where it is demonstrated that the bacteria which develop when sea water is confined in the laboratory are sufficient to arrest the growth and development of otherwise healthy larvae. The almost complete suppression of bacteria by the penicillin/streptomycin mixture does not, however, make all larvae do equally well. Other factors in the larvae, and perhaps in the water itself, are still at work. At one time the writer thought that the glycogen food reserves at the time of liberation might be of importance, but the investigations of Collyer (1957) showed that there was little difference between various batches of larvae examined.

The way in which the larvae and bacteria interact is unknown but there seem to be two possibilities: either certain bacteria may cause a disease in the larvae, or some or all of the normal bacterial constituents of the sea water may irritate the larvae either directly by settling and multiplying on the shell or flesh, or indirectly by the secretion of 'external metabolites' into the water.

Bacteria are sparse in the sea because, amongst other factors, of the relative absence of solid surfaces. A surface immersed in the sea, unless protected in some way, speedily becomes covered by bacteria. It has been suggested that because of the active physical properties of solid surfaces it is only at a surface that the dissolved organic matter, which is present in sea water in only very small quantities, reaches a high enough concentration to support a vigorous population (ZoBell, 1943). When the water is confined in a small vessel the area of surface becomes relatively large and the bacteria become very abundant. The smaller the containers the larger the bacterial population (ZoBell & Anderson, 1936). It is probable that many bacteria are associated with the larva both on the shell and perhaps on the surface of the living tissue. If the bacteria on the larvae are the important ones then this would give a mechanism whereby bacterial effects would be produced in the sea.

It can be seen from the results given in this paper and elsewhere (Walne, 1956) that batches of water differ considerably in the bacterial population which they will support when kept under standard conditions in the laboratory. If these differences are reflected in the bacterial population on the larvae in the field, then this could be a factor influencing the successful growth and development of marine larvae in the sea.

Whatever the mechanism, the results show a way by which the performance of a given batch of sea water can be improved in the laboratory. A number of workers in recent years have suspected that the dissolved organic matter in sea water plays an important part in the activity of marine animals. The observations of Collier, Ray, Magnitzky & Bell (1953) on the filtration of *Crassostrea* and of Loosanoff (1954) on rearing the larvae of *Crassostrea* and other species of lamellibranchs, may be cited. Of particular relevance are the papers of Wilson (1951) and Wilson & Armstrong (1952, 1954), in which the growth of *Echinus* larvae in waters of different origins are compared. If the results obtained by Wilson are viewed in the light of experiments reported in this paper, it seems possible that the bacterial floras, which waters from different areas will support, provide an explanation for Wilson's results. The flora will, in turn, depend on the nature and quantity of the organic compounds present in the water; little is known about these, but it is certain that they will vary considerably.

SUMMARY

Preliminary experiments had suggested that the extent of the development of the bacterial flora which develops when sea water is confined in small vessels is an important factor in the laboratory culture of oyster larvae. In the experiments reported in this paper the growth and settlement of oyster larvae was compared in controls of normal sea water with those in which the bacterial flora was controlled with antibiotics.

A concentration of 50 i.u. of the sodium salt of penicillin G per ml. suppressed bacterial growth for at least 2 days and, in a series of experiments using fifteen different broods of larvae, significantly ($P > 0{\cdot}001$) more spat

were obtained in those to which penicillin had been added than in the controls.

A mixture of 50 units of penicillin G and 0·05 mg streptomycin sulphate was tried in three series of experiments. This completely suppressed the development of bacteria for at least nine days. In all three series many more spat were obtained than in the controls, but in only one series was there more spat than in the comparable penicillin experiment. It is suggested that when the bacterial population is very vigorous, or perhaps when certain species are present, this mixture will be more useful than penicillin alone. Experiments with other concentrations of penicillin, and with streptomycin and chloromycetin alone were not successful.

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THE GROWTH RATE OF *VERRUCA STROEMIA* (O. MÜLLER)

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(Text-figs. 1 and 2)

The growth rate under both natural and experimental conditions of several littoral and sublittoral barnacles has recently been dealt with in some detail (Barnes & Powell, 1953, q.v. for earlier references; Barnes, 1952–53; Barnes & Barnes, 1954; Crisp, 1954; Barnes & Barnes, 1956; Barnes, 1956). No comparable data for *Verruca stroemia* are reported in the literature.

Although *V. stroemia* has been reported from the Red Sea (Darwin, 1854), the Adriatic (Kolosvary, 1947), and even the Indian Ocean (Nilsson-Cantell, 1938), the major area of its distribution is along the eastern Atlantic coasts of Europe from Spain to Finmark (for a review see Broch, 1924). In addition, Stephensen (1929, 1933) states that it is found in the coastal waters of Iceland, Greenland and the Faroes (see also Weltner, 1900, and Schaper, 1922). The species appears to be absent from the western Atlantic. Although recorded from a depth of 548 m by Weltner (1900), it is most common on relatively shallow banks and rough inshore grounds, and it extends into the lowest parts of the intertidal zone. According to Broch (1924) it is moderately euryhaline, but Schaper (1922) states that it does not occur in the Baltic Sea. In the Clyde Sea Area it is widely distributed on suitable stony ground from the lower littoral to deeper water and grows on crustaceans and molluscs. Although Broch considered the species to be lusitanian-boreal in character, with extensions into the Arctic, its breeding behaviour (as far as is known) suggests a cold-water origin; like many other boreo-arctic species, it has a single major brood each year that is liberated in the early spring.

THE MATERIAL AND METHODS

In previous studies on both *Balanus* and *Chthamalus* spp. the rostro-carinal axis along the base has been measured, but because of asymmetry this is less satisfactory with *Verruca stroemia*. The longest diameter at the base has, therefore, been measured and for brevity will be termed the length. For the small animals a binocular microscope with low-power objective and scaled ocular was used, and for the large, a measuring microscope with vernier attachment.

Animals were collected at an extreme low-tide level. For the estimation of annual growth under natural conditions large numbers of animals were measured and the results analysed with the help of probability paper. In order to estimate growth rates under conditions similar to those used for other species, stones bearing individuals well separated from each other were drilled and fastened to plastic panels; these were exposed on a raft at a constant depth of about 3 ft. Several such series were put out at different times of the year. At appropriate intervals the material was brought in, the animals carefully cleaned, and each one measured. Growth was followed for several years.

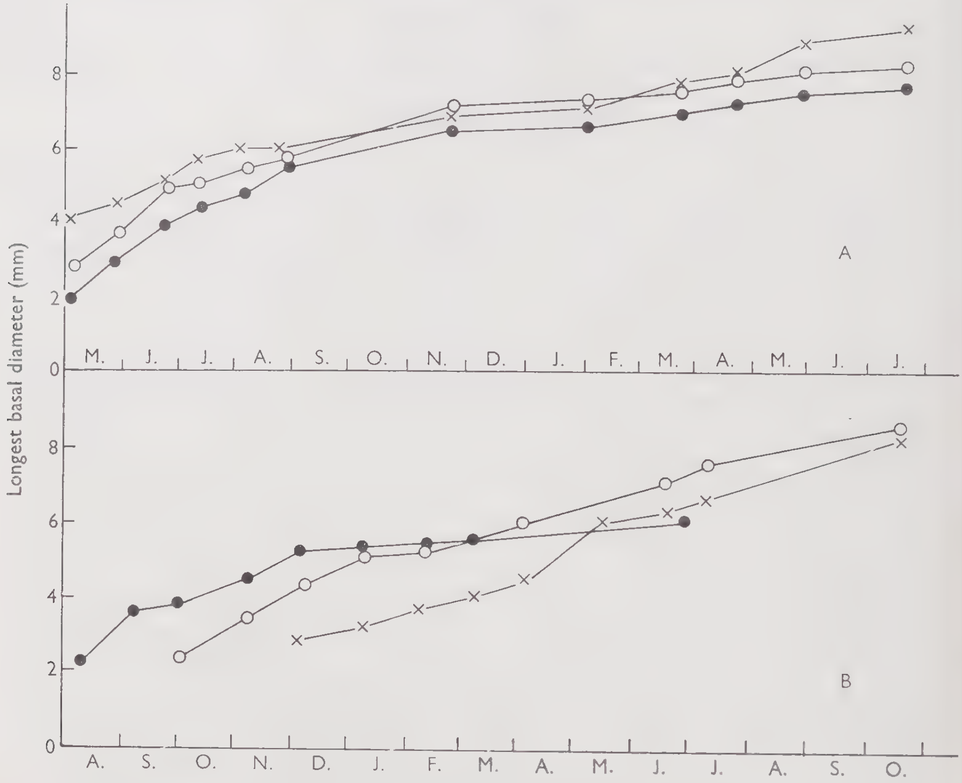

Fig. 1. Growth of *Verruca stroemia* over two fifteen-month periods under conditions of continuous immersion. Animals cleaned at each inspection. Exposures made throughout year. A, May 1955 to July 1956; ●—●, Series *a*; ○—○, Series *b*; ×—×, Series *c*. B, August 1956 to October 1957; ●—●, Series II; ○—○, Series III; ×—×, Series IV.

The mean length of each size-group and the mean specific growth rates (increase in length per unit length per day) have been caculated and are plotted in Figs. 1 and 2. In some series a number of animals were lost, particularly during the later stages of exposure, a fact that introduces some bias into the comparisons.

THE RESULTS

The first series was put out early in May 1955; it consisted of three size-groups of mean length 1·9, 2·8, and 4·1 mm., equivalent to first, second, and third year-groups on the shore (see p. 431). In all these growth was rapid and continuous during the late spring and the summer months. By late August there was little difference between the mean sizes of the three groups, which were 5·5, 5·7, and 6·0 mm.

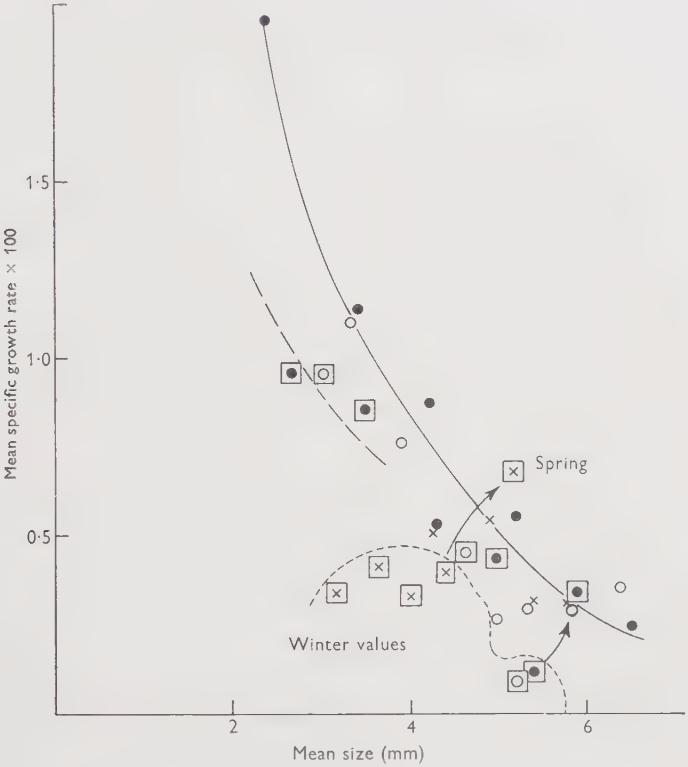

Fig. 2. The mean specific growth rate $\left(\frac{\Delta l}{\bar{l}.n}\right) \times 100$, of *Verruca stroemia*, for all series: symbols as in Fig. 1, values from Fig. 1 B in squares.

During the following early winter growth was slow, but it must be remembered that the animals were approaching their maximum size. Between the end of November and February of the next year there was no increase in size in any of the groups, but growth was renewed in the spring of 1956 and continued very slowly during the following summer. By the end of the second summer all the groups had reached their maximum size of about 8 mm. It is evident that under the experimental conditions, namely, exposure on a raft near the surface and freedom from epiphytic growth and mud, this sublittoral barnacle, which even extends into relatively deep inshore waters, grows rapidly. The maximum size is approached in only one season's growth—between settlement in the spring and the following November.

A second series, its mean size 2·2 mm (no smaller ones being available), was put out during the August of 1956. Growth took place rapidly and continued at about a uniform rate until early December when a size of 5·3 mm had been reached. During the following winter many of these animals were lost and the mean sizes are not representative of the whole series and cannot be compared with the values earlier in the season. However, inspection of the individual results shows that between January and June there was always a slight increase in size.

Series III was put out at a mean length of 2·4 mm in October 1956, that is again at the size reached in the second year under natural conditions. Growth took place almost uniformly throughout the winter months so that by January a mean size of 5·0 mm had been reached. Only a little growth took place during the late winter, but this was followed by a sudden increase in length during the following spring.

Series IV, with a mean length when exposed of 3·0 mm, was put out in December 1956; and it is evident from the graph that, although steady increments were added throughout the winter, growth was distinctly slow. In 4 months a mean size of only 4·6 mm had been reached, an increase that would have been attained in some 30 days during the spring months.

The mean specific growth rates for all three size-groups put out in May 1955 and followed for many months fall on a smooth curve, the values decreasing with increasing size (Fig. 2). There is no significant discontinuity that could be taken to indicate any marked seasonal effect. The second series, started in August, gives values that lie somewhat below this curve during the autumn and early winter. It is difficult to separate the two factors of the environment which are probably most important, namely, temperature and food, since the former tends to be minimal in the spring when food is maximal, and as the temperature rises the available food tends to decrease (although not necessarily below the value required for adequate metabolism) until the autumnal diatom outburst. The lower values in Series II, nevertheless, may be ascribed to inadequate food supply or, much less likely, to the deleterious effects of higher summer temperatures in the surface water as affecting particularly the smaller animals. The midwinter values of this series are only some 50% of the mean specific growth rates of Series I at the same size, and since the temperatures at this season are approaching those of the spring and early summer, it seems that the reduced growth rate if related directly to the environment is a result of inadequate food supply. This suggestion is substantiated by the fact that in the following spring a growth rate somewhat greater than that of the first series is found. Series III follows a similar pattern, the values for the autumn and early winter falling into line with those for

Series II (broken line); the midwinter values are again those of the first series and a return to the 'normal' is made in the following spring. Series IV was put out in December; the values are now initially very low, that is, throughout the winter, again supporting the hypothesis that food supply is a limiting growth factor at this time of the year. In the following late spring a much higher rate of growth was obtained at equivalent sizes than in Series I. This higher value in the spring and early summer in Series II and IV, and the fact that the effect is more marked in the smaller individuals, strengthens the suggestion that even the midsummer growth rates in Series I, although falling on a smooth curve, may be somewhat depressed by the higher summer temperatures or limited by food supply; however, loss of animals in the longer exposures tends to bias the results and the suggestion must be regarded as tentative.

The growth of individual animals was not followed on the shore. However, a large randomly taken population was measured during May and the frequency distribution plotted on probability paper. Three classes could be distinguished with mean lengths of 2·0, 2·8, and 4·0 mm. These seem to correspond to three consecutive year-groups—the first having settled in the early spring of the current year and having by May reached 2·0 mm, that is the size of the first exposures put out on the raft.

DISCUSSION

It is evident that *V. stroemia* follows a very similar growth pattern to several of the other species previously investigated. Settlement in the spring is followed by rapid growth; little increase in size takes place during the winter, but renewed growth is established in the following spring. The fact that animals exposed later in the year grow at almost the same rate as those put out in the spring at the same size suggests that there is no marked endogenous or seasonal rhythm *per se*. Growth appears to be directly related to conditions of environment, being rapid when there is abundant food. How far there is any seasonal change in growth rate in deep water, where the temperature range is small and food supply perhaps relatively so, is unknown. Indeed, it would be of considerable interest to have growth data from animals maintained in deep water under similar experimental conditions to the present series, in particular being kept free from encrusting organisms. Under these conditions any endogenous rhythm might be more easily recognized.

The rate of growth is very greatly increased under the experimental conditions and as with other species this may be ascribed in part to the greater availability of food in the surface layers and in part to the freedom from encrusting growths and mud—both common under natural conditions. The absence of the species from suitable substrata in shallow water cannot be ascribed to any deleterious effect of such an environment.

It is now possible to compare the growth characters of several species measured under the partially controlled conditions of raft exposure. For such a comparison the effect of size may be eliminated by using the specific growth rate and by employing its value at what may be termed the half life period, taken as half the maximum size attained by a species, we may expect to eliminate the effect of age. The values for several species are shown in Table 1.

TABLE 1

Species	Maximum size (mm)	Mean specific growth rate (× 100) at half size
Balanus balanoides	25	1·0
B. crenatus	25	1·3
B. balanus	43	0·9
Chthamalus stellatus	10	0·1
Verruca stroemia	8	1·0

The mean specific growth rates at half maximum size are, with the exception of *Chthamalus stellatus*, very similar, from which it may be assumed that the intrinsic metabolic activities are of the same order. Indeed, the apparent differences may well be the result of taking shell length as a measure of animal size and its rate of change. The value for *C. stellatus* is much lower than for the other species and while the above factor is again of importance a lower intrinsic metabolic rate is indicated. This agrees with the observations of Southward (1955) who found that over their optimal range the cirral beat of *C. stellatus* was slower than that of *Balanus balanoides*. However, unlike other species investigated, *Chthamalus stellatus* showed very little increase in growth rate when transferred to raft conditions; after an initial acceleration immediately following the change, the rate returned to a value little different from that on the shore (Barnes, 1956). Perhaps greater general stimulation—such as would be provided under natural conditions by wave action or experimentally by water currents—is necessary for the full metabolic activity of *C. stellatus*.

SUMMARY

Data are presented on the growth rate of *Verruca stroemia* under natural conditions and when exposed continuously and cleaned repeatedly. Several series exposed at different times of the year were followed.

Rapid growth takes place (under raft conditions) following settlement; the maximum size is virtually reached in one season's growth between spring and early winter. There is little growth in midwinter.

Differences between the mean specific growth rates of the various series can be ascribed to differences in the availability of food.

The question is discussed as to whether there is any seasonal rhythm; the evidence indicates that no marked rhythmic pattern of growth exists.

Observations on deep-water populations would be of value for comparison and to unmask any relatively weak rhythmic growth.

The mean specific growth rates at half their maximum size are compared for several species—*Balanus balanoides*, *B. crenatus*, *B. balanus*, *Chthamalus stellatus* and *Verruca stroemia*; it is similar for all species except *Chthamalus stellatus*. The high level barnacle may require stimulation such as is provided by wave action to elicit full metabolic activity.

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AN ADULT DIGENETIC TREMATODE FROM AN INVERTEBRATE HOST: *PROCTOECES SUBTENUIS* (LINTON) FROM THE LAMELLIBRANCH *SCROBICULARIA PLANA* (DA COSTA)

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(Plates I and II and Text-figs. 1 and 2)

Adult digenetic trematodes are typically parasites of vertebrates. Sexually mature trematodes occurring in invertebrate hosts are usually regarded as precocious last-larval stages, to which the term 'progenetic' is applied. These progenetic forms are often encysted, and can usually only contribute to the further life history of the species, and, in many cases, can only attain the definitive form of the adult trematode, after transference to a vertebrate host.

Specimens of the mud-burrowing lamellibranch *Scrobicularia plana*, collected from the region of the Thames estuary, were found to be infected with unencysted, sexually mature trematodes. These parasites occurred in the kidneys of the molluscs, and, as will be discussed later, there seems no reason to regard them as other than adult digenetic trematodes. They were identified as specimens of *Proctoeces subtenuis* (Linton, 1907) Hanson, 1950. This species has been recorded, hitherto, only as a parasite of the hind-gut of marine fishes belonging to the families Labridae and Sparidae, from the Red Sea, New Zealand, and the eastern seaboard of America.

In this paper an account is given of the occurrence, distribution, environment, morphology, and taxonomy of *Proctoeces subtenuis* from *Scrobicularia plana*, and the possible life history of this trematode in the Thames estuary is discussed with reference to the phenomenon of progenesis.

OCCURRENCE AND DISTRIBUTION

Specimens of *Proctoeces subtenuis* in the kidney of *Scrobicularia plana* were first observed in 1954 in animals collected from the mud flats at Chalkwell in Essex. During the subsequent three years nearly a thousand specimens of *S. plana* from this location were examined. Every single specimen was infected. The degree of infection, estimated from examination of fifty-one hosts, varied from one to thirteen parasites per host, with an average of between four and five. There appeared to be no correlation between degree of infection and size of host.

The distribution of *S. plana* at Chalkwell has already been discussed by Freeman & Rigler (1957). The mollusc is plentiful at mid-tide level, but absent from the sandy deposits lower down the shore. It lies in a burrow 6–10 in. below the surface of the mud, maintaining contact with the overlying water through long, extensible siphons. Spooner & Moore (1940) record population densities of *S. plana* of up to 1000 per m^2 in the Tamar estuary at Plymouth, and Green (1957) gives 500–1025 per m^2 as the population density in the Gwendraeth estuary in South Wales. The impression derived from collecting at Chalkwell on numerous occasions is that similar population densities occur there also. No attempt has been made to delimit the occurrence of the parasite along the Thames estuary to the east and west of Chalkwell, but infected specimens of *S. plana* have been collected from stations over a distance of about a mile of the mud flats in the neighbourhood of Chalkwell. The total population of *S. plana* in this area must number many millions, and the evidence is consistent with the view that all of them are infected with the trematode. The only occurrence from a locality other than Chalkwell was at Whitstable in Kent where three specimens of *S. plana* of about 150 examined were infected. Specimens of *S. plana* have been examined from the Rivers Tamar and Tavy (*ca.* 80), the Gwendraeth (*ca.* 100), the Rivers Dart and Teign in south Devon (*ca.* 40), the Butley river in Suffolk (7), the Essex rivers Blackwater (*ca.* 50) and Colne (7), and Conway in North Wales (*ca.* 100), but no trematodes have been found. Specimens of the lamellibranchs *Macoma balthica* (L.) and *Mya arenaria* L., which occur alongside *Scrobicularia plana* in the mud flats at Chalkwell, have also been examined, but were found to be uninfected.

In addition to living specimens of *Proctoeces subtenuis*, the kidney of infected *Scrobicularia plana* was observed often to contain dead parasites. These varied in colour between light brown and almost black, and also varied in texture, the darker specimens being harder and even stone-like. These dead specimens clearly showed the external form of *Proctoeces subtenuis*, the oral and ventral suckers being evident, and, in some cases, the cirrus was extruded (Pl. I, fig. 1). The dead specimens occurred in about one in ten of all infected *Scrobicularia plana* examined, and as many as six were found in a single host. They always occurred together with living trematodes in the same host. It is known that some nematode parasites become calcified after death (von Brand, 1952), and a phenomenon comparable to that described above has been reported for another trematode by Macfarlane (1939).

ENVIRONMENT OF THE PARASITE

Proctoeces subtenuis has previously been reported as a parasite of the hind-gut of certain marine fishes. The present work is concerned with the occurrence of this trematode in the kidney of an estuarine lamellibranch. It is very probable that the conditions of the immediate external environment of the parasite in such different situations, in such different hosts, are widely dissimilar. A brief account will therefore be given of some aspects of the environmental conditions of a parasite within the lamellibranch kidney, with particular reference to the kidney of *Scrobicularia plana*.

The lamellibranch kidney consists essentially of a proximal, glandular, ciliated tubule (the organ of Bojanus) and a ureter which conveys the excretory products to the supra-branchial space, and thence to the outside world. The organ of Bojanus receives fluid through a ciliated coelomostome from the pericardium. This fluid is derived from the blood by ultra-filtration through the heart wall. The tubular filtrate is isotonic with the blood, but differs slightly from blood in its ionic composition (Robertson, 1949). Modification of the filtrate occurs in the tubule by the addition of nitrogenous excretory compounds, of which urea and the amino acids taurine and creatine have been identified in twenty-two lamellibranchs (Letellier, quoted by Delaunay, 1927). In the freshwater mussel, *Anodonta cygnea*, as much as 91·6% of the total nitrogen in the tubular fluid can be protein nitrogen, and this can represent a concentration of eight times the blood plasma protein nitrogen level (Florkin & Duchateau, 1948).

The infected population of *Scrobicularia plana* at Chalkwell is exposed to considerable seasonal variations in the tonicity of the overlying water, and these variations are accompanied by corresponding changes in the tonicity of the blood (Freeman & Rigler, 1957). Since the tubular filtrate in other lamellibranchs has been shown to be isotonic with the blood, it seems likely that the osmotic pressure of the tubular fluid will vary with changes in external salinity. This was examined by equilibrating specimens of *S. plana* to sea water and to 70% sea water, and, using the method described by Freeman & Rigler, comparing the depression of the freezing-point of fluid drawn directly from the organ of Bojanus with that of the external medium. No significant difference was found between the osmotic pressure of the tubular fluid and that of the external medium.

It seems certain, therefore, that the trematodes are exposed to an environment which is variable in osmotic pressure, and, in this respect, they resemble free-living estuarine turbellarians (Krogh, 1939) rather than adult trematode parasites in vertebrate hosts. It also seems possible that the fluid in which the trematodes live in the kidney of *S. plana* has an electrolyte composition similar to that of sea water, but has a high concentration of proteins and/or amino acids.

DESCRIPTION OF THE PARASITE

Size and shape.

Proctoeces subtenuis is generally cylindrical, but tapers at each end. The mouth is subterminal, and the ventral sucker is situated at about one-third of the total body length from the anterior end. The integument is without spines. Although living specimens are cylindrical, the general practice in studies of the Digenea of illustrating a well-flattened specimen has been followed here (Text-fig. 1). Some measurements, in mm, of ten such specimens, randomly chosen from a collection of permanent preparations, are given in Table 1. The first figure given is an arithmetic mean for the sample, and the figures in parentheses indicate the range for the sample. The egg capsule measurements were made on samples of five eggs from each of twenty adults, i.e. 100 eggs in all.

TABLE 1. MEASUREMENTS OF *PROCTOECES SUBTENUIS*

See text

	Millimetres
Length	2·92 (1·52 – 4·80)
Maximum width	0·96 (0·56 – 1·40)
Oral sucker length	0·20 (0·14 – 0·32)
Oral sucker width	0·21 (0·14 – 0·30)
Prepharynx length*	0·03 (0·01 – 0·05)
Pharynx length	0·15 (0·10 – 0·19)
Pharynx width	0·15 (0·12 – 0·25)
Oesophagus length	0·22 (0·01 – 0·45)
Ventral sucker length	0·34 (0·24 – 0·57)
Ventral sucker width	0·39 (0·26 – 0·61)
Anterior end of body to anterior border of ventral sucker	0·90 (0·40 – 1·40)
Cirrus sac length	0·54 (0·42 – 0·70)
Cirrus sac maximum width	0·13 (0·10 – 0·20)
Anterior testis diameter	0·19 (0·10 – 0·28)
Posterior testis diameter	0·21 (0·12 – 0·32)
Germarium diameter	0·16 (0·05 – 0·27)
Egg capsule length	0·042 (0·026 – 0·073)
Fgg capsule width	0·024 (0·015 – 0·030)

* Visible in only seven of the ten specimens in the sample.

When freshly collected specimens of *P. subtenuis* were transferred to dishes of sea water, they became quite active, but the muscular contraction and stretching was confined almost entirely to the region of the body anterior to the ventral sucker (Pl. 1, figs. 2A, B). In contracted specimens the genital pore was situated approximately midway between the oral and ventral suckers, but in stretched specimens it was that region anterior to the genital pore that was most elongated, so that the genital pore came to lie relatively nearer to the ventral sucker. This variation assumes special significance when it is considered that the position of the genital pore has sometimes been used as a diagnostic character of some digeneans.

Colour

Perhaps the most immediately noticeable feature of the specimens of *P. subtenuis* from the kidney of *Scrobicularia plana* is that they are red or pink. This coloration is not obviously restricted to any particular organ-system of the trematode but is generally distributed. The intensity of the colour varies with the size of the animal; even specimens about 1 mm long are noticeably pink, and large (3–4 mm) specimens are distinctly red. As is shown in Table 4, *Proctoeces* species have previously been described from the hind-gut of fishes on fourteen occasions, and in none of these descriptions was it observed that the specimens were red in colour. The name *Proctoeces erythraeus*, given by Odhner (1911) to a species from the Red Sea, is apparently a reference to the geographical location, rather than to the colour, of this trematode (cf. *Tomopteris erythraea* Caroli, 1928).

Red coloration in several other trematodes is known to be due to the presence of haem pigments (von Brand, 1952). Haem pigments react with certain nitrogenous compounds to form the appropriate haemochromogen, the absorption bands of which can be recognized by spectroscopic examination. Observations were made with a microspectroscope on specimens of *Proctoeces subtenuis* mounted in sea water in an attempt to characterize the pigment. No absorption bands could be recognized on examination of untreated specimens, but, after addition of Takayama's fluid (Hawk, Oser & Summerson, 1954), the characteristic absorption bands of pyridine haemochromogen were readily observed. The α-band at about 558–560 mμ was particularly evident, and a less well-defined β-band at about 525 mμ was also observed. Pieces of tissue taken from the host, *Scrobicularia plana*, including the wall of the kidney and the brown-coloured pericardial gland, gave no indication of the formation of pyridine haemochromogen when treated with Takayama's fluid.

The red coloration of specimens of *Proctoeces subtenuis* inhabiting the kidney of *Scrobicularia plana* is, therefore, due to a native haem pigment, whereas there is no evidence that members of the same species inhabiting the hind-gut of fishes are similarly pigmented.

It is not possible, on the basis of the present information, to suggest whether this pigment plays any part in the respiratory processes of the parasite, nor why it should be developed by specimens in the kidney of *S. plana* whilst apparently being absent in specimens in the hind-gut of fishes. It is, however, interesting to note that the only other adult platyhelminth parasite occurring in *S. plana*, the viviparous rhabdocoele *Paravortex scrobiculariae* (Graff), is red in colour (Freeman, 1957), whereas the closely related species *P. cardii* Hallez, occurring in cockles and some other lamellibranchs, is colourless. It is hoped in the near future to be able to determine whether this red coloration in *P. scrobiculariae* is also due to a haem pigment. A study of this assumption of a red coloration by platyhelminth parasites of *Scrobicularia plana* might throw some light on the metabolism of this lamellibranch as well as on that of the parasites.

Alimentary canal.

The mouth opens into the oral sucker which is succeeded by a pre-pharynx, which in turn communicates with the pharynx. In whole mount preparations, the posterior end of the oral sucker often overlies the anterior end of the pharynx, thus completely obscuring the pre-pharynx (Pl. I, fig. 5A), but in sections the pre-pharynx is always readily evident (Pl. I, fig. 4). Posterior to the pharynx is the oesophagus, and the junction between these two regions receives the openings of a ring of gland cells. The oesophagus itself is provided with well-developed circular and longitudinal muscles, and, depending on the state of contraction of these muscles, the length of the oesophagus may vary very considerably. Thus, in some whole mount preparations the oesophagus may appear long (Pl. I, fig. 5B), and in others very short (Pl. I, fig. 5A), and so the presence or absence of the oesophagus, based on examination of whole mount preparations, is particularly unreliable as a diagnostic character. All the regions of the alimentary canal mentioned so far, namely the oral sucker, pre-pharynx, pharynx and oesophagus, are lined by a cuticle (Pl. I, fig. 4), which is continuous with that covering the external surface of the body. Posterior to the oesophagus the alimentary canal bifurcates into two simple intestinal caeca which reach almost to the posterior end of the body. This intestinal region is lined by an epithelium of columnar cells, each with a basal nucleus (Pl. II, fig. 4A). In living specimens the alimentary canal invariably contains an abundance of refractile globules (Pl. II, fig. 3) that readily take up fat stains and blacken with osmium tetroxide, but the kidney tissue of the host lacks such droplets.

Excretory system.

The most prominent feature of the excretory system is the Y-shaped bladder (Pl. II, fig. 1), the paired arms of which extend as far forwards as the posterior limit of the pharynx. Posteriorly, at about the level of the testes, these arms join to form the median stem that runs backwards as a relatively wide tube. Peristaltic waves were observed to pass posteriorly along this median stem of the excretory bladder. The median stem narrows posteriorly to join a cuticular-lined terminal duct (Pl. II, fig. 5), the junction receiving the ducts from a surrounding collar of gland cells (Pl. II, fig. 5). Similar glands have been illustrated in other trematodes, but their function appears not to be known. Except in its extreme posterior region, the Y-shaped excretory bladder lies in the dorsal half of the body in a plane immediately ventral to the alimentary canal. The anterior extremities of the bladder lie to the outside of the oesophagus, then, as they run posteriorly, they pass beneath the intestinal limbs to appear on the inside of these structures at about the level of the germarium.

Numerous tributary ducts open into the anterior extremities of the arms of the Y, at least two prominent vessels on each side coming from the anterior region of the trematode, and one on each side from the posterior region. The tributary vessels were best seen in well-flattened living specimens (Pl. II, fig. 3) and were very difficult to observe in fixed specimens whether sectioned or mounted whole. Attempts were made to inject pigmented latex through the excretory pore in order to trace the tributary vessels but these attempts were unsuccessful.

In sections near to the anterior limits of the excretory bladder, the tributary ducts appear to have walls composed of 'fibrillar cytoplasm', i.e. they are non-cellular, and thus differ from the walls of the bladder itself, which are lined by a cellular epithelium (Pl. II, figs. 4A–C). The bladder epithelium, as seen in sections, consists of somewhat hemispherical or conical cells with large nuclei (Pl. II, fig. 4B). The cytoplasm often contains an abundance of refractile spherical bodies about 1–2·5 μ in diameter (Pl. II, fig. 4C), which readily take up haematoxylin, and, only a little less readily, acid stains such as eosin and Orange G. These spherical bodies are insoluble in xylene and do not take up Sudan dyes or blacken with osmium tetroxide. They may also occur freely in the lumen of the excretory bladder. The fibrous nature of the walls of the tributary ducts was described for *Proctoeces ostreae* by Fujita (1925), who also observed, 'la paroi de la vessie consiste en une très mince membrane pourvue de noyaux et de corpuscles d'excrétion, en particulier dans la paroi des rameaux'.

The excretory system of *P. subtenuis* (and also apparently of *P. ostreae*) assumes a special taxonomic significance in view of La Rue's (1957) recent classification of the Digenea based on the structure of the wall of the excretory bladder. La Rue divides the Digenea into two super-orders, which he characterizes as follows—Anepitheliocystidia: 'primitive excretory bladder retained, i.e. not replaced by cells from mesoderm, hence definitive bladder not epithelial'; and the Epitheliocystidia: 'primitive excretory bladder surrounded by, and then replaced by, layer of cells derived from mesoderm, hence definitive bladder thick walled and epithelial'. The Fellodistomatidae (which includes *Proctoeces*) is placed in the Anepitheliocystidia, its position there being thought to have been firmly established by the work of Cable. However, as described above and illustrated in Pl. II, fig. 4A–C, the excretory bladder in our specimens of *P. subtenuis* is lined by a well-developed epithelium.

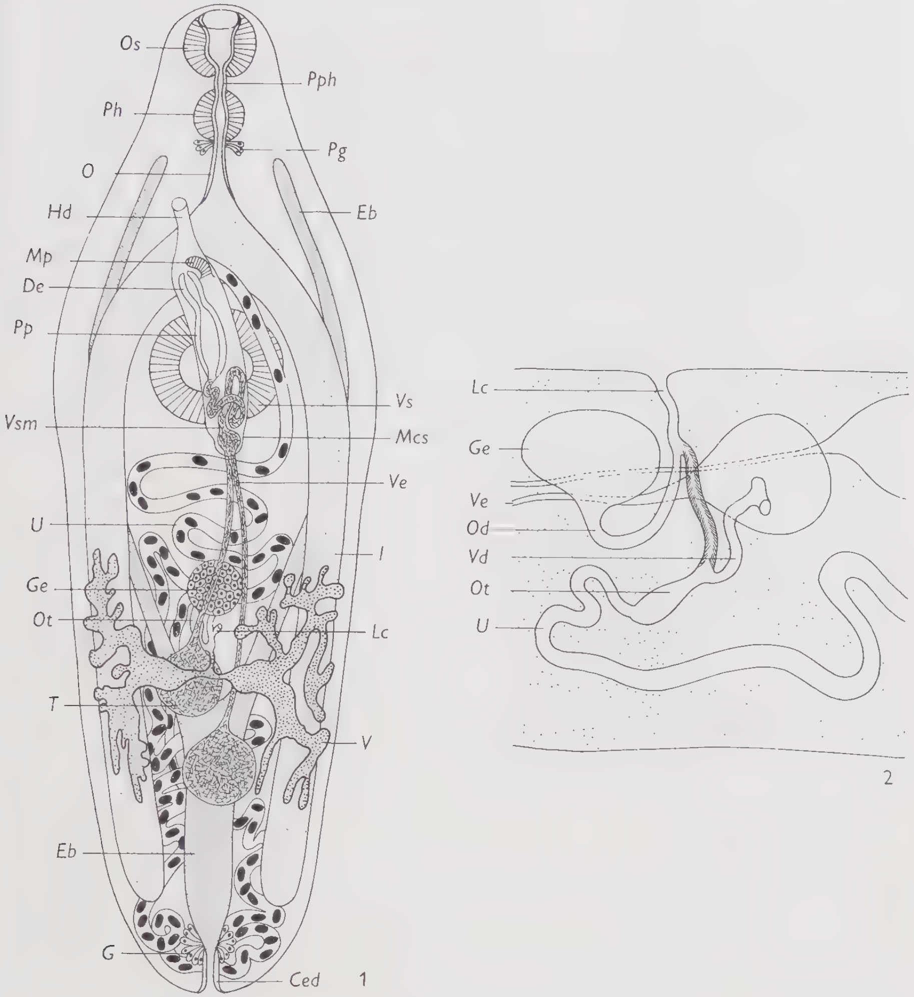
Occasionally the excretory bladder of *P. subtenuis* contains about five to twenty spherical bodies (Pl. II, fig. 2), each about 50 μ (35–66 μ) in diameter, and which in section may be seen to consist of several concentric, radially striated layers. These bodies are stained intensely by haematoxylin. Similar structures have been recorded from the excretory bladder of other trematodes (von Brand, 1952).

Male genitalia (Text-fig. 1)

The two spherical unlobed testes are situated in slightly oblique tandem in the anterior region of the posterior third of the body. Each testis gives rise anteriorly to a vas efferens, the two ducts running forwards fairly directly and joining with each other at their point of entry into the cirrus sac. When at rest this sac lies longitudinally in the body and overlies the ventral sucker, overlapping it both anteriorly and posteriorly, but pressure during histological processing may force it to lie entirely behind or in front of the ventral sucker. The posterior end of the cirrus sac is situated near the sagittal plane of the worm, but anteriorly it curves a little to the left to become confluent with the uterus to form the hermaphrodite duct.

The united vasa efferentia (there is practically no vas deferens) lead immediately to a much-coiled vesicula seminalis which may fill the posterior half of the cirrus. In ten of a sample of twenty flattened specimens the posterior region of the vesicula seminalis was somewhat conical as illustrated in Text-fig. 1, but in the remaining ten specimens such a conical region was not evident. It is possible that in living specimens a proximal conical region of the vesicula seminalis is demarcated, but that it may become obliterated during histological preparation.

The vesicula seminalis leads anteriorly to a 'pars prostatica' which is surrounded by unicellular glands, and which contains villous projections into its lumen. The pars prostatica leads in turn to a muscular ejaculatory duct, and this opens into the relatively narrow hermaphrodite duct. Active sperms were seen in the ejaculatory duct of pressed living specimens, even in specimens less than 1 mm long which contained no egg capsules. Lying sometimes alongside and sometimes anterior to the ejaculatory duct is a 'muscular papilla' (Pl. I, fig. 3A, B; Text-fig. 1). When lying alongside the ejaculatory duct, this papilla forms part of the wall of a pouch that is completed by the ejaculatory duct itself (Text-fig. 1). Such a muscular papilla has been reported in other species of *Proctoeces* (Fujita, 1925; Yamaguti, 1934, 1938), and a somewhat similar structure was described in the gasterostome *Bucephalopsis tylosuris* by Ozaki & Ozaki (1952) as a 'genital tongue'. By applying pressure to the cover-glasses of whole mount preparations of living *Proctoeces subtenuis*, the cirrus was sometimes caused to extrude, and, during this extrusion, the muscular papilla was seen to lie anteriorly to the muscular end of the ejaculatory duct. The muscular papilla preceded the cirrus proper in its passage out of the cirrus sac and was of such size as apparently forcibly to enlarge the normal diameter of the cirrus sac and hermaphrodite duct. The function of this muscular papilla is not known, but its topographical relationships and intimate association with the male intromittent organ suggest that it could play some part in the 'geometrical fitting' of co-copulants, as has been shown by Ullyot & Beauchamp (1931) to occur in certain turbellarians.



Text-figs. 1, 2. *Proctoeces subtenuis.* Text-fig. 1. Whole animal in dorsal view. (Diagram based on microprojections of whole mount preparations, with adjustments so that dimensions agree with the mean measurements included in Table 1.) Fig. 2. Proximal regions of the genitalia in side view. (Reconstructed from serial longitudinal sections.) *Ced*, cuticular-lined terminal excretory duct; *De*, ductus ejaculatorius; *Eb*, epithelial-lined excretory bladder; *G*, gland cells surrounding junction of epithelial- and cuticular-lined regions of excretory system; *Ge*, germarium; *Hd*, hermaphrodite duct; *I*, intestine; *Lc*, Laurer's canal; *Mcs*, muscular wall of cirrus sac; *Mp*, muscular papilla; *O*, oesophagus; *Od*, oviduct; *Os*, oral sucker; *Ot*, ootype; *Pg*, pharyngeal glands; *Ph*, pharynx; *Pp*, pars prostatica; *Pph*, prepharynx; *T*, testis; *U*, uterus; *V*, vitelline follicles; *Vd*, vitelline duct; *Ve*, vas efferens; *Vs* ventral sucker; *Vsm*, vesicula seminalis.

Female genitalia (Text-figs. 1, 2).

The germarium is slightly smaller than either of the testes, and lies just in front of the anterior testis, about one-third of the total distance between this organ and the ventral sucker. From a mid-ventral position the germarium gives off the oviduct which then runs posteriorly before bending dorsally to give off the Laurer's canal. There is no receptaculum seminis. The Laurer's canal opens on to the mid-dorsal region of the body between the germarium and the anterior testis. After giving off the Laurer's canal, the oviduct passes postero-ventrally to a position ventral to the anterior border of the anterior testis, where it receives a median duct from the vitelline system. The part of the oviduct between the origin of the Laurer's canal and the point of entry of the vitelline duct is lined with proximally directed cilia, i.e. the cilia would be capable of promoting a current along the oviduct from the vitelline duct to the Laurer's canal. A correspondingly ciliated region of the oviduct has been reported in some other digeneans, e.g. *Coitocaecum anaspidis* by Macfarlane (1939).

The vitellarium is irregularly follicular (Pl. II, fig. 6), and is situated mainly in the peripheral regions of the body. It is generally distributed between the anterior border of the germarium and the posterior border of the posterior testis, but in several specimens it extended beyond these limits in both directions. The collecting ducts from the vitelline follicles are not well defined and it is often difficult to identify even the transverse duct, which, in other trematodes, is usually prominent. Again, in some specimens the median longitudinal duct leading to the oviduct is recognizable only after sectioning, but in others it is full of vitelline cells and would merit the description 'vitelline reservoir'.

Immediately after receiving the vitelline duct, the oviduct bends ventrally and anteriorly through nearly 180° in a sagittal plane, and widens to pass forwards as the ootype. Anteriorly, the ootype narrows again to become the uterus, which soon bends on itself to pass backwards to the posterior region of the worm. Here it becomes coiled in the ventral half of the body before eventually passing anteriorly, finally to take a fairly straight course alongside or beneath the cirrus sac, to open into the hermaphrodite duct ventral to the cirrus.

The hermaphrodite duct is provided with strongly muscular walls and leads to the genital pore, which, in fixed specimens, lies midway between the oral and ventral suckers to the left of the mid-line of the body (Text-fig. 1).

Egg capsules.

In a sample of 176 specimens of *Proctoeces subtenuis* from forty-one hosts, all but one of the trematodes had egg capsules in the uterus. This single specimen was very small, measuring 0·72 mm when flattened and fixed, and, although no egg capsules were present, the vesicula seminalis contained active sperms. The larger specimens each contained at least one to two thousand capsules.

The egg capsules were oval, and about twice as long as wide ($42 \times 24\ \mu$). In some cases there was considerable variation in the size of capsules. Those measured for Table 1 varied in length from 26 to 73 μ, but there were only three longer than 52 μ. The capsules were light brown, with relatively thin, elastic walls which were capable of being stretched by muscular movements of a contained embryo. No operculum could be seen, even in capsules which contained active embryos and which had been freely liberated by the trematode. Furthermore, when capsules were pressed under cover-glasses until they burst, the lines of fracture did not reveal any incipient operculum. It was, however, possible to recognize a polarity in most capsules, a small part of the wall being thickened to form a disc at that end nearest to the posterior end of the embryo.

In a sample of twenty specimens of *P. subtenuis*, collected from hosts that contained more than one parasite per host, all twenty were found to have egg capsules enclosing active embryos. Only one of over 200 specimens of *Scrobicularia plana* examined contained only a single trematode, and, although this parasite possessed numerous egg capsules, they contained no embryos.

Attempts to obtain embryos freed from their capsules were unsuccessful. Capsules which had been freely liberated from the trematodes were kept in sea water for periods of up to 7 days, but no hatching took place. Pressure was applied to the capsules in an attempt to release the embryos, but, although the capsules were ruptured, the miracidia were always damaged. Therefore, it was possible to make observations on embryos only while they were still enclosed in their capsules. The embryos (Pl. II, fig. 7) were seen to be ciliated, and ciliary activity was sometimes manifested by a rotation of the embryo alternately to the left and right about the long axis, and also by the agitation of hyaline droplets, presumably residual yolk material, lying freely in the capsule, particularly around the equator of the embryo. The orientation of the cilia suggested that the posterior end of the embryo lay near to the thickened disc in the capsule wall. A constant feature to be seen at the other end of the embryo was a prominent refractile body. The only other internal structures that could be identified were two flame cells situated about mid-way along the length of the embryo. It is possible, however, that more flame cells may have been present.

Evidence was obtained that egg capsules are normally liberated by the trematodes while in the kidney of *S. plana*. Twenty-five hosts were carefully examined and egg capsules were found lying freely in the kidneys of three of them. Two of these hosts contained two capsules each, and the third over 500. A further twenty-five hosts were kept in separate dishes of filtered sea water and the contents of the dishes examined daily. Free egg capsules were found in two of them. An estimate of the rate of egg laying was obtained by isolating freshly collected trematodes in filtered sea water at 19° C, and making daily counts of the egg capsules in each dish. The numbers of eggs laid on each of 5 days after isolation are given in Table 2. It is possible that eggs liberated immediately after removal of the trematode from the host were forcibly discharged as a result of mechanical stimulation involved in the act of removal, but the figures in the table show that eggs continued to be liberated for several days while the trematodes were left undisturbed.

TABLE 2. RATE OF EGG LAYING OF *PROCTOECES SUBTENUIS*

Days after isolation ...	1	2	3	4	5
Specimen A	123	9	10	5	8
B	17	0	Specimen died		
C	1	0	0	14	Died
D	37	8	0	0	7
E	82	3	51	4	5

It may thus be concluded that this parasite regularly produces large numbers of fertile eggs, and that these are liberated into the kidney and pass outside the body of the host. The ciliation of the embryo suggests the occurrence of a free miracidial stage, but there was no evidence of a special operculum to facilitate hatching. It is interesting to note the observation by Baer (1952, p. 127): Curiously enough, this ciliated coating is also found in miracidia that remain within the egg and hatch only when the latter is swallowed by the snail.'

The eggs are enclosed in a thin, light-brown capsule. The hardening of trematode egg capsules has recently been reviewed by Johri & Smyth (1956), and may be summarized as follows:

> Dihydroxy-phenols are oxidized, through the agency of a phenol-oxidase, to the corresponding quinone, which combines with protein to form a tanned protein or sclerotin. The histochemical techniques used by Johri & Smyth have demonstrated that the vitellaria are the site of the protein, phenol, and phenol-oxidase involved. Stains such as malachite green have an affinity for the vitellaria and are believed to be specific for the protein component localised there. Diazo-reagents, such as Fast Red B, are specific for phenolic substances, and the localization of these phenols can also be demonstrated by use of ammonium molybdate. The presence of a phenol-oxidase acting on certain dihydroxy-phenols can be demonstrated by incubating alcohol-fixed specimens at 37° C for about 2 h in catechol, protocatechuic acid or dihydroxy-phenyl-alanine (DOPA).

These techniques were applied to specimens of *Proctoeces subtenuis*, and also, for comparison, to *Gastrocotyle trachuri*, a monogenetic trematode from the gills of the horse mackerel *Trachurus trachurus*. A specimen treated with Fast Red B is shown in Pl. II, fig. 6. The results (Table 3) refer in each case to the intensity of reaction localised in the vitellaria.

These show the presence in the vitellaria of *Proctoeces subtenuis* of the phenol and protein components of the egg tanning processes, but negative results were consistently obtained for techniques which, under identical conditions, demonstrated the presence of a phenol-oxidase in *Gastrocotyle*.

TABLE 3. EGG TANNING PROCESSES OF *GASTROCOTYLE TRACHURI* AND *PROCTOECES SUBTENUIS*

For explanation see text

	G. trachuri	*P. subtenuis*
Malachite green	+	+
Fast Red B	++	++
Ammonium molybdate	++	+
Catechol	++	o
Protocatechuic acid	++	o
DOPA	++	o

The use of catechol, protocatechuic acid, and DOPA as substrates will necessarily reveal only the occurrence of an enzyme acting on these dihydroxy-phenols, which may possibly be derived from tyrosine. Pryor (1955) has suggested the role of amino-phenols derived from some other source, probably tryptophan, in the hardening of some dipterous puparia. Perhaps a similar mechanism may be operative in *Proctoeces subtenuis*.

TAXONOMY OF THE PARASITE

Our description clearly identifies this trematode as belonging to the genus *Proctoeces* Odhner, 1911, of the family Fellodistomatidae, subfamily Haplocladinae. Yamaguti (1953) lists seven species of *Proctoeces*, together with a larva of which specific identification was not possible. Winter (1954) proposed another new species. Several of the species are based on very few specimens, sometimes only one, and their distinction rests on differences of relative body proportions and on egg size. We could not, however, refer our trematodes exclusively to any one of these species. Several hundred specimens were examined, and it is apparent that many of the characters thought to indicate specific differences probably represent intraspecific variations of the kind emphasized by Stunkard (1957). The present species are *P. maculatus* (Looss, 1901) Odhner, 1911; *P. erythraeus* Odhner, 1911; *P. subtenuis* (Linton, 1907) Hanson, 1950; *P. insolitus* (Nicoll, 1915) Dollfus, 1952; *P. ostreae* Fujita, 1925; *P. major* Yamaguti, 1934; *P. magnorus* Manter, 1940; and *P. macrovitellus* Winter, 1954.

Proctoeces was established by Odhner (1911) for *Distomum maculatum* Looss, 1901 and *Proctoeces erythraeus*. He distinguished *P. erythraeus* by its smaller ventral sucker (at least one-third smaller), its smaller eggs, and the shorter extent of the vitellaria. These differences are best illustrated by comparing the following measurements, in mm, taken from Odhner (1911):

	P. maculatus	*P. erythraeus*
Length	2·5	3·0
Ventral sucker	0·42 – 0·70 × 0·28 – 0·42	0·38 × 0·40 (about 0·3 in life)
Vitellaria	Extend behind posterior testis	Extend to anterior border of posterior testis
Eggs	0·072 – 0·079 × 0·027	0·045

Dawes (1946) lists *P. erythraeus* as a synonym of *P. maculatus* and says (p. 245) that only the difference of egg size is significant, 'but this is marred by the fact that only a solitary mature specimen was found'. *P. erythraeus* was reinstated by Manter (1947) who found six specimens in *Calamus* spp. at Tortugas, Florida. He gave the egg size as 46–53 × 19–24 μ and concluded that 'the extent of the vitellaria varied some but never reached past the posterior testis as it does in *P. maculatus*'.

Hanson (1950) collected two trematodes from *Calamus* sp. at Bermuda which agreed with *Proctoeces erythraeus* in sucker ratio, extent of vitellaria, and egg size, but she argues that Linton's (1907) description of *Distomum subtenue*, also collected from *Calamus* sp. at Bermuda, agrees with *Proctoeces erythraeus* sufficiently for them to be considered the same species. Linton's measurements, in mm, of two specimens of '*Distomum subtenue*' are:

Length	3·6	2·07
Ventral sucker	0·68	0·30 × 0·48
Eggs	0·05 × 0·02	0·042 × 0·015

He illustrates the vitellaria extending behind the posterior testis. Whereas Hanson's two specimens were said to agree with *Proctoeces erythraeus* in all three characters of egg size, sucker ratio, and extent of vitellaria, one of Linton's specimens has a considerably larger ventral sucker (the measurement of 0·68 mm was from a living specimen) and the vitellaria extend behind the posterior testis. Our material from *Scrobicularia plana* showed that the vitellaria could stop short of the posterior testis in some specimens while extending behind it in others. Examination of only a few specimens would permit a conclusion that would not have been true for all the specimens available. It seems best, therefore, to recognize the general similarity of *Distomum subtenue* and *Proctoeces erythraeus*, particularly because of identical hosts in Bermuda, and regard them as the same species, *P. subtenuis* (Linton, 1907) (syn. *P. erythraeus* Odhner, 1911), as was proposed by Hanson.

Comparison of *P. erythraeus* (as described by Odhner, Manter and Hanson) and *Distomum subtenue* (as described by Linton) illustrates the amount of individual variation within the same species. It also shows the difficulty of reaching any firm statement of differences between related species. On the evidence of size of ventral sucker and extent of vitellaria at least one of Linton's specimens agrees with Odhner's description of *Proctoeces maculatus*. This adds emphasis to Dawes's (1946) observation that only the difference of egg size is significant in separating *P. maculatus* from *P. subtenuis* (= *P. erythraeus*). The average egg dimensions (42 × 24 μ) of our trematodes agree with those of *P. subtenuis*, but the largest egg measured (73 × 30 μ) is within the size range given by Odhner for *P. maculatus*. While it seems generally true that the characters of sucker size, extent of vitellaria, and size of eggs are sufficient to distinguish *P. maculatus* and *P. subtenuis*, intraspecific variation may lead to some overlap in all three characters. Odhner, the only author who had the opportunity of directly comparing *P. maculatus* and *P. subtenuis* (=*P. erythraeus*), considered them to be separate species. On the evidence available then it seems advisable to follow Odhner, but a critical re-examination of numerous specimens of these two species is obviously desirable.

Nicoll (1915) collected five trematodes from the rectum of *Sparus australis* and named them *Xenopora insolita* n.g., n.sp. This species was later placed in *Proctoeces* by Dollfus (1952). In egg size, ventral sucker size, and extent of vitellaria, *P. insolitus* agrees with *P. subtenuis*, but differs in the extension of the hermaphrodite duct a short distance behind the ventral sucker, the situation of the cirrus pouch entirely behind the ventral sucker, and in the vesicula seminalis being mainly external to the cirrus pouch. Odhner comments that in Looss's specimens of *P. maculatus* the cirrus sac was displaced behind the ventral sucker by pressure, the genital sinus (hermaphrodite duct) being stretched to twice its normal length. In some of our preparations of *P. subtenuis* the cirrus sac was similarly situated almost entirely behind the ventral sucker. Differences in extent of the hermaphrodite duct and the position of the cirrus sac are not in themselves, therefore, sufficient specific characters. The extension of the vesicula seminalis outside the cirrus sac is contrary to Odhner's diagnosis of *Proctoeces* and, as Yamaguti (1953) observes, this feature of *P. insolitus* requires confirmation. In all other respects *P. insolitus* agrees with *P. subtenuis*, but, pending further examination of the vesicula seminalis, it is felt that *P. insolitus* must remain a separate species.

The specific status of *P. ostreae* Fujita, 1925, has been discussed by Dollfus in his addenda to Fujita's paper. He concludes that it is a progenetic metacercaria that has not yet attained complete maturity nor definitive size, and declines to come to any firm conclusion whether it is a separate species or merely a metacercaria of *P. maculatus*. Its validity must therefore remain in doubt.

P. major Yamaguti, 1934, shows some resemblances to *P. maculatus*, but differs significantly in the size of the body, the trilobate shape of the ovary, and the size of the eggs. To these might be added the position of the genital pore to the right of the mid-line, not, as usually in *Proctoeces*, to the left.

The final species in Yamaguti's list is *P. magnorus* Manter, 1940, based on one specimen from Cerros Island, Mexico. Manter considered it most similar to *P. erythraeus* as described by Odhner (1911), but differing in having a larger oral sucker and smaller eggs. He says that it is also probable that *P. magnorus* differs from *P. erythraeus* in having shorter vitellaria, an acetabular stalk, a shorter oesophagus, a cirrus sac not reaching the ovary, and a longitudinal groove within the acetabular cavity. It is difficult to know how Manter reached these conclusions, since Odhner nowhere describes or figures the oral sucker, oesophagus, or cirrus sac of *P. erythraeus*. In view of the variability we have shown in extent of vitellaria and in egg size it would seem unwise to base a new species on such characters when only a single specimen has been examined. *P. magnorus* must, therefore, be considered a synonym of *P. subtenuis* (syn. *P. erythraeus*).

P. macrovitellus Winter, 1954, was based on two specimens from the posterior part of the intestine of *Cymatogaster aggregatus* Gibbons from Southern California. This was the first record of *Proctoeces* from a fish of the family Embiotocidae, and also the first from the west coast of America. Many of the features of *P. macrovitellus*, as described and figured by Winter, are in disagreement with the original generic diagnosis by Odhner (1911) and the modified diagnosis by Yamaguti (1953). *P. macrovitellus* has the genital pore to the right of the mid-line at the level of the pre-pharynx, the vitelline follicles

TABLE 4. A REVISED LIST OF THE SPECIES OF THE GENUS *PROCTOECES* AND THEIR FISH HOSTS

Host	Family	Location	Authority
P. maculatus (Looss, 1901)			
Labrus merula L.	Labridae	Trieste	Looss, 1901
Julis pavo (L.) (= *Crenilabrus pavo*)	Labridae	—	—
Symphodus (= *Crenilabrus*) *griseus* (Gmelin)	Labridae	—	—
Blennius ocellaris L.	Blenniidae	Naples	Odhner, 1911
Milio (= *Sparus*) *macrocephalus* (Basilewsky)	Sparidae	Inland Sea	Yamaguti, 1934
Sparus sarba Forskål (= *S. aries*)	Sparidae	—	—
Pagrosomus auratus (Bloch & Schneider)	Sparidae	—	—
Epinephelus aka-ava Bleeker	Serranidae	—	—
Semicossyphus reticulatus (Cuvier & Valenciennes)	Labridae	Tarumi	Yamaguti, 1938
Crenilabrus sp.	Labridae	Black Sea	Wlassenko, 1931
Duymaeria flagellifera (Cuvier & Valenciennes)	Labridae	Hamazima	Yamaguti, 1953
P. subtenuis (Linton, 1907)			
Calamus calamus (Cuvier & Valenciennes)	Sparidae	Bermuda	Linton, 1907
Bodianus rufus (L.) (= *Harpe rufa*)	Labridae	—	—
Iridio bivittata (Bloch)	Labridae	—	—
Lachnolaimus maximus (Walbaum)	Labridae	—	—
Calamus sp.	Sparidae	Bermuda	Hanson, 1950
Latridopsis ciliaris (Forster)	Latridae	Wellington, N.Z.	Manter, 1954
P. subtenuis (but described as *P. erythraeus*)			
Sparus (= *Chrysophrys*) *bifasciatus* (Forskål)	Sparidae	Red Sea	Odhner, 1911
Thalassoma lunare (L.) (= *Julis lunaris*)	Labridae	—	—
Calamus calamus (Cuvier & Valenciennes)	Sparidae	Tortugas	Manter, 1947
C. bajanado (Bloch & Schneider)	Sparidae	—	—
P. subtenuis (but described as *P. magnorus*)			
Caulolatilus anomalus (Cooper)	Sparidae	Mexico	Manter, 1940
P. insolitus (Nicoll, 1915)			
Sparus australis (Günther)	Sparidae	North Queensland	Nicoll, 1915
P. major Yamaguti, 1934			
Pagrosomus auratus (Bloch & Schneider)	Sparidae	Tarumi	Yamaguti, 1934
Unknown species or species of doubtful validity; in molluscan hosts			
P. ostreae Fujita, 1925			
Ostrea gigas Thunberg	Ostreidae	Hiroshima	Fujita, 1925
Proctoeces larva, Yamaguti, 1938			
Brachiodontes senhausi (Reeve)	Mytilidae	Lake Hamana	Yamaguti, 1938

situated wholly in front of the testes, thick-shelled eggs, and the cirrus sac not parallel to the long axis of the body but acutely twisted in the first third of its length. Winter also describes and figures an ovoid receptaculum seminis, measuring 0·079 by 0·095 mm, situated immediately in front of the ovary. The presence of a receptaculum seminis would, in itself, exclude Winter's species from *Proctoeces* as defined hitherto. We consider that Winter's species cannot be included in *Proctoeces* without drastic revision of the limits of this genus as at present understood.

A revised list of the species of *Proctoeces*, based on the foregoing systematic considerations, with the modern names of their hosts, is given in Table 4. It is now possible to assign the trematodes from *Scrobicularia plana* exclusively to the species *Proctoeces subtenuis* (Linton, 1907).

DISCUSSION

There seems little doubt from their morphology that our specimens of *Proctoeces subtenuis* are adult digenetic trematodes. They exhibit, in all significant features, the same structure as other members of the species described from fish hosts, and the availability of very large numbers has, indeed, allowed a more detailed account of the structure and variability of this species than was hitherto possible.

All possible adult forms of this trematode have been found, from the small immature form, through larger sexually mature individuals, to dead specimens present in the same kidney with living individuals. Their unencysted condition in the kidney makes copulation and fertilization possible: the only specimen found singly in a host had undeveloped eggs. There is evidence that eggs containing active, ciliated miracidia are liberated from the trematode and passed out of the host. Such eggs were found free in the kidney and also in dishes in which infected hosts had been isolated. The eggs presumably pass from the kidney by the urinary pore into the supra-branchial space and reach the exterior by the exhalant siphon.

The very heavy infection of *Scrobicularia plana* at Chalkwell, and the isolated occurrences of the trematode at Whitstable, must have been initiated, and must be maintained, by larval stages developing in an invertebrate host. The life history of at least one member of the Fellodistomatidae is known (Palombi, 1934), and a cercaria undoubtedly belonging to this family has been described by Martin (1945) and redescribed, without reference to Martin's work, by Cable (1954). In both, the cercaria was non-ocellate and trichocercous, and developed in sporocysts in a marine lamellibranch. The life cycle studied by Palombi was of *Bacciger bacciger* (Rud.), subfamily Fellodistomatinae, and Martin's cercaria can almost certainly be referred to the same subfamily. *Proctoeces* belongs to the subfamily Haplocladinae, and Cable (1953) suggests that the cercariae of this subfamily may be furcocercous or trichofurcocercous. We have found furcocercous cercariae developing in sporocysts in *Scrobicularia plana* at Chalkwell, but the fact that they occurred also in *S. plana* from the Gwendraeth and Tavy rivers where *Proctoeces subtenuis* was absent suggested that they were not related to this trematode. This impression that adult and cercarial stages of *P. subtenuis* did not both occur in *Scrobicularia plana* was confirmed by the negative results of numerous attempts to infect *S. plana* with these furcocercous cercariae, and the failure to find post-miracidial stages in *S. plana* into which fertile eggs of *Proctoeces subtenuis* had been introduced.

There is some evidence that *Proctoeces* in Japanese waters has an unencysted metacercaria in marine lamellibranchs. As already noted, the trematode described by Fujita (1925) as *P. ostreae* from the gonad of the edible oyster of Japan, *Ostrea gigas*, was considered by Dollfus to be a metacercaria of an undetermined species of *Proctoeces*, and similarly Yamaguti (1938) described a single immature stage of *Proctoeces* sp. from the digestive gland of the mytilid *Brachiodontes senhausi*. The normal life cycle of *Proctoeces* thus appears to be as follows: adult trematodes live in the hind-gut of labrid or sparid fishes; cercariae, which may be furcocercous, trichofurcocercous or trichocercous, develop in sporocysts in marine lamellibranchs; and unencysted metacercariae also occur in marine lamellibranchs. These metacercariae are almost certainly transferred to the fish which is the definitive host when it eats the lamellibranch (Yamaguti, 1938).

Our evidence indicates that this life cycle of *Proctoeces* must be modified for *P. subtenuis* in British waters. The apparent limitation of the species to the region of the Thames estuary, in a lamellibranch widely distributed in estuarine muds around the British Isles, strongly suggests that the introduction of *P. subtenuis* to this country is fairly recent. It is typically a parasite of fish of Asian, Australasian or eastern American waters. It could possibly have arrived through the accidental introduction of a normal fish host, or, more probably, in the marine lamellibranch that is the first intermediate host. The cercariae issuing from this mollusc could have entered *Scrobicularia plana* and, now being outside the range of the normal fish host, have achieved maturity in the kidney of this lamellibranch. It may be noted that a trichocercous cercaria has been found in the lamellibranch *Petricola pholadiformis* Lamarck at Whitstable (M. Duval, personal communication). *P. pholadiformis* is a recent introduction from the east coast of North America (Newell, 1954), and has a limited distribution in the southern and eastern parts of Britain, including the Essex coast of the Thames estuary (Cole, 1903). Work is now being done to determine whether the cercaria from *P. pholadiformis* is a stage in the life cycle of *Proctoeces subtenuis* in the Thames estuary. The evidence so far available suggests that the fish host has been eliminated from the life cycle. No sparids have been recorded from the Thames estuary, and only two members of the Labridae occur there, namely *Labrus bergylta* Ascanius (three specimens up to

1903 (Laver, 1903; Murie, 1903)), and *Crenilabrus melops* (L.) which is common. Any final statement on the life cycle of *Proctoeces subtenuis* in the Thames estuary must, of course, include consideration of the only common labrid in the area, but transference of the trematode from its last molluscan host to a fish must almost certainly be effected by the fish eating the mollusc, and there is no evidence that a lamellibranch such as *Scrobicularia plana* is eaten by *Crenilabrus melops*. The nature of the habitat of *Scrobicularia plana* makes it unlikely that it is readily available as food for fish, although shells have occasionally been found in the stomach of some flatfish (J. E. Forrest, personal communication). The limitation of *Proctoeces subtenuis* to the mouth of the Thames also suggests that a fish host is not involved in the life cycle. At Chalkwell, every specimen of *Scrobicularia plana* examined was infected, and yet the trematode appears to be absent from *S. plana* in the neighbouring Essex rivers, the Blackwater and Colne. It seems unlikely that mobile fish, acting as hosts to breeding trematodes, would not have produced a wider distribution of the parasite while the intensive infection of *S. plana* at Chalkwell was taking place.

If this view is correct then the trematodes in *S. plana* are directly comparable, structurally, functionally, and in their status in the life history, with the adults found in the hind-gut of fishes in other parts of the world. They enter the kidney of *S. plana* as immature stages, achieve maturity there, and eventually die there. They certainly differ from the stages of *Proctoeces* previously described from lamellibranchs. The single immature stage described by Yamaguti (1938) from *Brachiodontes senhausi*, and termed by him an adolescaria, had undeveloped female genitalia. Dollfus relegated Fujita's *Proctoeces ostreae* to the status of progenetic metacercariae on the grounds that only a few eggs were present, even these being abnormal, and that the animals did not appear to have attained complete maturity nor definitive size. In these respects our *P. subtenuis* clearly agrees more closely with previous descriptions of any of the adults of this trematode from fish hosts than with any metacercarial stage of related species. There seems no reason to modify the emphasis on their adult nature by invoking the phenomenon of progenesis.

The term progenesis was first used by Giard & Bonnier (1887) to describe the assumption of sexual maturity by male parasitic isopods while still in a larval condition. It has since been applied to numerous digenetic trematodes which become sexually mature as cercariae or metacercariae in an intermediate host. Many examples are listed by Wu (1938) and Dawes (1946). The only significant question from the point of view of this study is that posed by Dollfus (1929) in the title of his paper: 'Existe-t-il des cycles évolutifs abrégés chez les trématodes digénétiques?' This question is further discussed by Joyeux, Noyer & Baer (1930) and again by Wu (1938). Wu concludes that 'up to the present we still have no definite proof that there is an abridged life cycle or omission of a final host in the life history of a digenetic trematode'.

This proof was provided by Serkova & Bychowsky (1940). They exposed uninfected *Bithynia tentaculata* (L.) to members of the same species of snail which contained mature *Asymphylodora progenetica*, and obtained experimental infections. They identified rediae and also mature adults in the previously uninfected snails. In this abbreviated life cycle the rediae develop in one specimen of *Bithynia tentaculata* and the adults achieve maturity in another specimen of the same species. Serkova & Bychowsky assume that an alternative life cycle of *Asymphylodora progenetica* is possible, in which the parasite of the mollusc enters a fish and there attains sexual maturity. The importance of Serkova & Bychowsky's work, in relation to our study of *Proctoeces subtenuis*, lies in the precedent it established that abbreviated life cycles of digenetic trematodes are possible. More recently, Buttner (1955) has shown that *Ratzia joyeuxi* (Brumpt, 1922) and *Paralepoderma brumpti* (Buttner, 1950), both normally parasites of snakes, can show abbreviated life cycles in which the final host is omitted.

We suggest that the facts presented in this paper provide strong circumstantial evidence that *Proctoeces subtenuis* in the Thames estuary displays a similar phenomenon of a life cycle restricted to invertebrate hosts. A final decision on this question must, however, await the results of further investigations.

Most of the observations reported in this paper were carried out at the Plymouth laboratory and we are grateful to the Director and his staff for many kindnesses shown to us during the course of this work. We are particularly indebted to Dr J. S. Alexandrowicz for much assistance with the literature. We wish to thank Mr N. B. Marshall of the British Museum (Natural History) for help in compiling the list of fish hosts, and also Miss Emily Clay, Dr J. Green, Mr S. V. N. Casey and Mr B. W. Jones for sending specimens of *Scrobicularia plana* from localities we were unable to visit.

SUMMARY

The digenetic trematode *Proctoeces subtenuis* (Linton, 1907) is recorded from the kidney of the lamellibranch *Scrobicularia plana* (da Costa). At Chalkwell in Essex all of nearly a thousand *S. plana* examined were found to be infected, as were three of approximately 150 *S. plana* at Whitstable in Kent. The average number of parasites per host was between four and five. No *Proctoeces subtenuis* were found in *Scrobicularia plana* examined from other localities in Essex, Devon, Wales and Suffolk. The environmental conditions of a parasite in the lamellibranch kidney are discussed. It was found that the kidney fluid surrounding the parasites varies in osmotic pressure.

The anatomy of the parasite is described. The excretory bladder has a cellular wall, thus disagreeing with the inclusion of *Proctoeces subtenuis* in La Rue's superorder Anepitheliocystidia. The red colour of the parasite is due

to a native haem pigment. The egg capsules are hardened by a quinone-tanning mechanism, but incubation with catechol, protocatechuic acid, and dopa failed to reveal the presence of a phenol-oxidase.

The species of *Proctoeces* are reviewed, and some revisions suggested. *P. magnorus* Manter, 1940 is considered a synonym of *P. subtenuis*, and the synonymy of *P. erythraeus* Odhner, 1911 with *P. subtenuis* is confirmed. The differences between *P. insolitus* (Nicoll, 1915) and *P. subtenuis*, and between *P. maculatus* (Looss, 1901) and *P. subtenuis*, require re-examination. It is considered that *P. macrovitellus* Winter, 1954 should be excluded from the genus *Proctoeces*. A definitive host list of *Proctoeces* spp. is given.

The adult nature of *P. subtenuis* from *Scrobicularia plana* is discussed, and it is suggested that *Proctoeces subtenuis* in the Thames estuary shows an abbreviated life cycle restricted to invertebrate hosts.

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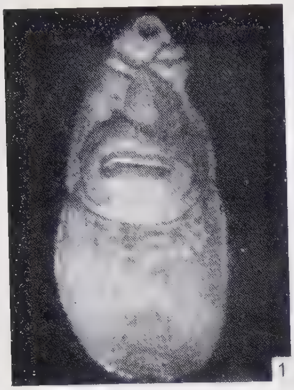
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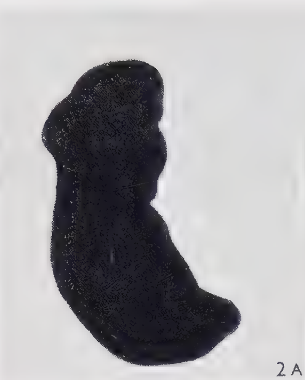
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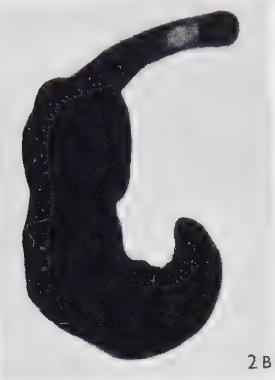
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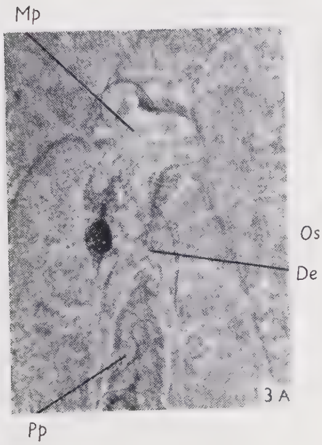

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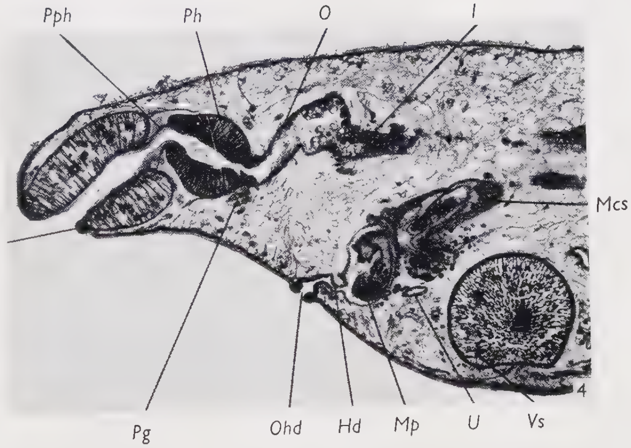
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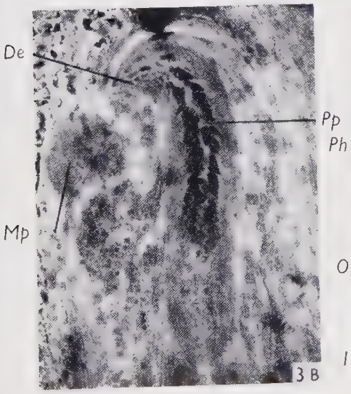

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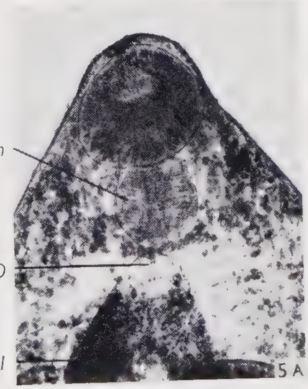

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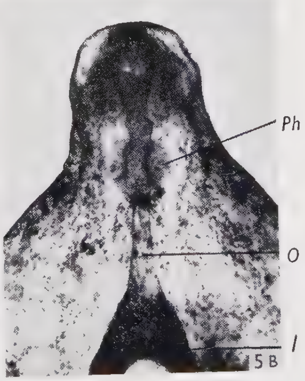

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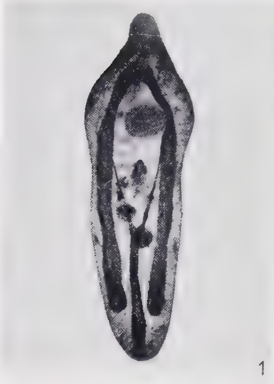
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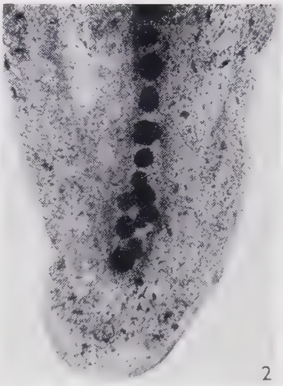
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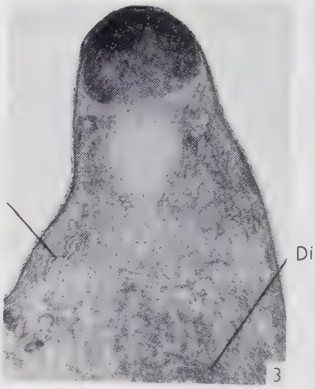

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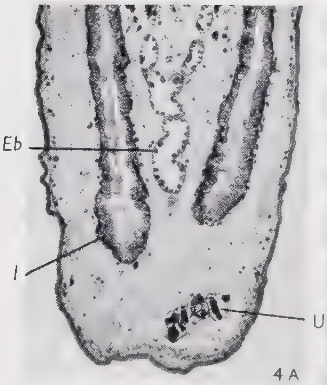

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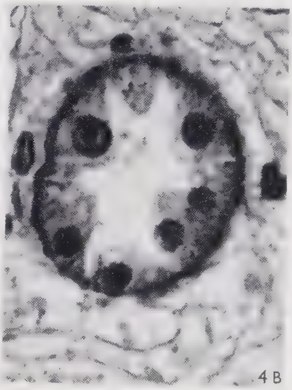
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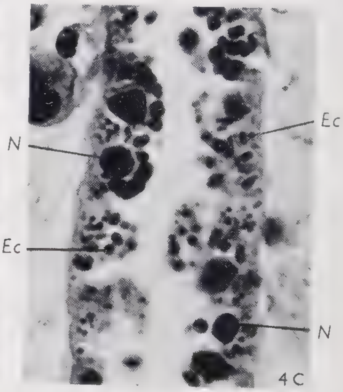

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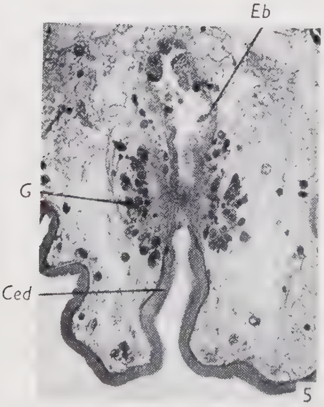

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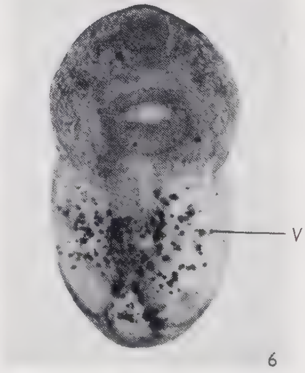

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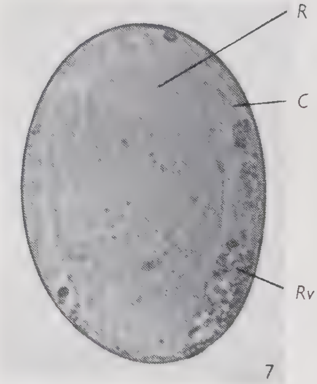

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EXPLANATION OF PLATES

Proctoeces subtenuis. Unless stated otherwise, all photomicrographs were made from fixed specimens mounted in Canada Balsam.

PLATE I

Fig. 1. Dead, 'petrified' specimen (unmounted). Fig. 2. (A) Contracted specimen (living). (B) Same specimen as (A), stretched. Fig. 3. Terminal part of cirrus, showing muscular papilla. (A) Living specimen. (B) Another specimen stained and mounted in Canada Balsam. Fig. 4. Vertical longitudinal section (slightly oblique) through anterior region. Fig. 5. Variation in length of the oesophagus in fixed preparations. (A) Contracted. (B) Stretched (different specimen from (A)).

PLATE II

Figs. 1–5. The excretory system

Fig. 1. The Y-shaped excretory bladder (from a preparation by Miss P. Milsom). Fig. 2. Concretions in the posterior region of the bladder (haematoxylin preparation). Fig. 3. Tributary canals in anterior region (living). Note also the droplets in the intestine. Fig. 4. The epithelial lining of the excretory bladder. (A) Horizontal section. (B) Transverse section. (C) Longitudinal section through epithelial cells charged with 'excretory corpuscles'. Fig. 5. Junction of epithelial-lined portion of bladder with terminal cuticular-lined portion, surrounded with gland cells (horizontal section). Fig. 6. The vitellaria. (Specimen treated with a diazo reagent to show phenolic substances.) Fig. 7. Egg capsule with ciliated embryo. (Living specimen, well pressed beneath cover-glass.)

Abbreviations

C, cilia; *Ced*, cuticular-lined terminal excretory duct; *De*, ductus ejaculatorius; *Di*, droplets in intestine; *Eb*, epithelial-lined excretory bladder; *Ec*, 'excretory corpuscles'; *G*, gland cells surrounding junction of epithelial- and cuticular-lined regions of excretory system; *Hd*, hermaphrodite duct; *I*, intestine; *Mcs*, muscular wall of cirrus sac; *Mp*, muscular papilla; *N*, nucleus; *O*, oesophagus; *Ohd*, opening of hermaphrodite duct; *Os*, oral sucker; *Pg*, pharyngeal glands; *Ph*, pharynx; *Pp*, pars prostatica; *Pph*, pre-pharynx; *R*, refractile body at anterior end of embryo; *Rv*, residual vitelline material; *Td*, tributary duct of excretory system; *U*, uterus; *V*, vitelline follicles; *Vs*, ventral sucker.

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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

X. SEASONAL CHANGES IN OXYGEN CONSUMPTION

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Marine Station, Millport

(Text-figs. 1–5)

The oxygen consumption of *Calanus* has been studied by various workers. They give figures which, although not precisely the same, are of the same order of size. From these figures can be calculated the food requirements and these may be expressed as a percentage of its weight per day or as the volume of sea water of known richness which it must filter each day. However, a copepod does not feed continuously; when carrying out diurnal vertical migration it feeds chiefly during the hours when it is in the surface layers (Gauld, 1953). Again, the overwintering Stage V *Calanus* do not migrate vertically (Nicholls, 1933), but stay in deep water and at this time phytoplankton is very scarce. Altogether then, the volume of 72 ml., calculated as necessary for a *Calanus* to filter daily when the sea is moderately rich in phytoplankton (Fuller & Clarke, 1936), must be an underestimate for long periods of the year.

Many experiments have been made to find the volume which a *Calanus* actually does filter daily, with very varying results, from the 1 ml. of Fuller (1937) to the 250 ml. of Harvey (1937). From the decrease of diatom numbers between cruises in the North Sea, Cushing (1955) has calculated that a *Calanus* may filter as much as 1500 ml. daily.

Recently, using cultures labelled with radioactive phosphorus, we concluded (Marshall & Orr, 1955) that the volume is often below 80 ml. daily. Confronted with this gap between food required and food available, it seemed desirable to re-investigate oxygen consumption.

METHODS

In the earlier work two methods were used for the measurement of respiration. In the first (Marshall, Nicholls & Orr, 1935; Clarke & Bonnet, 1939) the *Calanus* were kept at constant temperature in dark bottles with 1–4 ml. of water to each copepod, and the oxygen consumed over a period of about 4 h measured by the Winkler method. In the second (Clarke & Bonnet, 1939; Raymont & Gauld, 1951) respiration was measured with a constant pressure manometer using *Calanus* in concentrations the same as, or higher than, in the first method. These high concentrations were necessary to obtain a suitable reduction in oxygen in a time short enough for bacterial metabolism to be negligible.

The concentration in these experiments was high and the effect of crowding was tested by comparing the oxygen consumption of the same catch of ripe female *Calanus* in two parallel experiments, one using sixty *Calanus* in bottles of 60–70 ml. capacity for 4 h at 10° C and the other, single *Calanus* in bottles of about 30–40 ml. at 10° C. In the first (four bottles) the oxygen used, expressed as μl./*Calanus*/h, ranged from 0·336 to 0·392 and averaged 0·367; in the second (nine bottles) the range was from 0·220 to 0·348 μl./*Calanus*/h and the average 0·304. The crowding may therefore have affected the oxygen consumption slightly. On the other hand, when the concentration was changed from 10 in a 35 ml. bottle to 3, there was only a small difference in respiration. Another experiment comparing 100 *Calanus* in 160 ml. bottles with single *Calanus* in 60 ml. bottles for a longer time gave similar results.

By the use of antibiotics the bacterial population can be controlled so that an experiment may be allowed to last a much longer time. According to Oppenheimer (1955) penicillin is one of the most effective in sea water, but unfortunately it reacts with the iodine liberated in the Winkler method for oxygen determination and so it could not be used. We therefore used a mixture of equal parts of streptomycin and chloromycetin which were both found by Oppenheimer to be very effective in concentrations of 50 mg/l. With these, only slight changes in the oxygen content of a control sample of sea water occurred over a period of up to a fortnight. At the end of this time there was usually a sudden breakdown of the antibiotic effect and a correspondingly sharp fall in the oxygen content of control bottles. In some instances moulds also developed. Antibiotics are best added to the filtered sea water at least 10 h before an experiment since the oxygen content may fall appreciably during this period, thereafter remaining constant at the lower level.

The method eventually adopted was to keep three *Calanus*, which had been picked out from townettings taken the previous day, for 2 days in a bottle of 30–40 ml. capacity at 10° C, which is within the annual temperature range in the sea for this latitude. The water used was taken directly from the sea and passed through a membrane filter of about 1 μ average pore diameter and the antibiotics added (50 mg/l. of each). If sufficient numbers were available, ten experimental bottles were used for each stage and four or five controls.

Respiration was measured on Stage V, ripe and unripe females of both the *finmarchicus* and the *helgolandicus* forms, males, and young stages as each was available. In the early experiments Stage V and female *Calanus* were not distinguished as *finmarchicus* or *helgolandicus* but these forms were later (from October, 1956) tested separately and, from July 1956, ripe females were separated from immature, the intermediate stages of maturity being discarded. From July 1956 also the carapace length of all specimens used was measured. These measurements were made after the copepods had been exposed to alkali and acid in the oxygen determination and, although comparable among themselves, may differ slightly from those of formalin-fixed specimens.

It was not possible to follow any stage throughout a complete year. From the end of October to the end of February ripe *finmarchicus* females were absent whereas during the summer months immature females were very scarce. The *finmarchicus* form is predominant throughout the spring and summer, the *helgolandicus* form during the autumn. Stage V copepodites of both forms were absent during March.

Smaller bottles and larger numbers of individuals were used for the younger stages. For nauplii and even for Copepodites I and II the bottles had to be very small (2–10 ml.) to get a measurable reduction in the oxygen and this was determined by the method of Fox & Wingfield (1938). On the suggestion of Dr M. R. Droop a slight improvement was made in the method. After the reagents are sucked up into the barrel of the pipette, a small bead of mercury is introduced by a half turn of the milled head. This acts as a 'stirrer' when the pipette is shaken and gives a more uniform and finely divided precipitate. Duplicate samples then give much closer results.

The early nauplii can be readily obtained from eggs allowed to hatch in the laboratory; they develop up to Nauplius III without requiring food (Marshall & Orr, 1956). The later nauplius stages, as well as the earlier copepodites, were picked out from the plankton but only small numbers were obtained.

One difficulty, particularly with the small individuals, was that if one animal only was used, the oxygen reduction was little beyond experimental error, whereas if several were used some were likely to moult and so give less reliable results. The figures from those bottles in which a significant proportion had moulted (except with nauplii) or died, were rejected.

Several hundred Nauplius I or II were used at a time but it was difficult to ensure that all those counted were actually put in the experimental bottle; some were always lost with the insertion of the stopper. The count at the end was therefore used for calculation. Since Nauplii I and II usually moult within 24 h, the majority moulted during the course of an experiment and the first point on the curve (Fig. 2) therefore represents Nauplii I and II.

EXPERIMENTAL WORK

It seemed possible that the metabolism of *Calanus* might be reduced in winter not only by the lower temperature but also by the decrease in food supply, and the winter be spent in a state of 'hibernation'. To test this, Stage V *Calanus* captured in winter were kept singly at constant temperature (usually 10° C) in bottles of about 70 ml. capacity. The *Calanus* were first kept in the laboratory overnight to avoid the fall in respiration which occurs during the first 24 h after capture (Marshall *et al.* 1935). A large number of bottles was set up and the oxygen consumption measured every 3 or 4 days by removing eight or ten bottles and drawing off samples for analysis. The oxygen consumption varied considerably from one individual to another but on the whole it was decidedly lower than the earlier published results. Each of the experiments lasted for a little over 2 weeks but there was no indication that respiration decreased with time. The earlier figure taken for Stage V at 10° C was 0·25 μl./*Calanus*/h, but in these long-term winter experiments the average was 0·14.

Experiments were continued into the spring months and the respiration then began to rise, reaching a figure of 0·560 μl./*Calanus*/h for immature females at 10–14·5° C. At first it was thought that the high value might be caused by bacterial infection. In the following winter further experiments were made by the standard method using antibiotics and were carried on more or less regularly throughout the year. Once again one or two very high results were obtained in the spring and it then became clear that there was a seasonal variation in oxygen consumption.

TABLE 1. FALL IN RESPIRATION WITH TIME

1956			1957		
Total hours	O_2 μl./*C.*/h for total	Calculated μl./*C.*/h for period	Total hours	O_2 μl./*C.*/h for total	Calculated μl./*C.*/h for period
69·5	0·508	0·508	47	0·591	0·591
141·5	0·506	0·504	96	0·493	0·399
213	0·376	0·119	192	0·495	0·497
293	0·342	0·251	288	0·431	0·303
357·5	0·304	0·130			

The seasonal changes are shown in Fig. 1 and Table 2. In 1956 the respiration of females (Fig. 1B) was low at the beginning of April. There was then a rapid rise and values remained high until the middle of May. By June they had fallen to near winter values again. During spring and summer females were almost certainly *C. finmarchicus* and mostly ripe. In the following year the spring rise was earlier. It lasted from the beginning of April to the middle of June when there was a rapid fall.

During the spring of 1956, when respiration was near its peak, twenty-five female *Calanus* were kept singly in 70 ml. bottles and their oxygen consumption measured at 3-day intervals, to show if the high figure would be maintained. Control samples of sea water were analysed at the same time. Consumption remained high for the first 6 days and then fell. If allowance is made for the initial period of high respiration, it can be shown (Table 1) that the fall is considerable and that the respiration reached winter values by the end of the experiment. This was repeated with similar but less striking results in 1957.

The results for Stage V, immature female *C. finmarchicus* and males from October 1956 to late 1957 are shown in Fig. 1C. For males most values are

TABLE 2. OXYGEN CONSUMPTION OF ADULT AND STAGE V *CALANUS*

At 10° C in experiments lasting 48 h. The measurement in the second column represents length.

	Ripe females						Unripe females						Males			Stage V					
	C. finmarchicus			*C. helgolandicus*			*C. finmarchicus*			*C. helgolandicus*						*C. finmarchicus*			*C. helgolandicus*		
	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h	No.	mm	µl./C/h
1956																					
18–20. vii.	12*	—	0·296	—	—	—	11*	—	0·241	—	—	—	—	—	—	—	—	—	—	—	—
24–26. vii.	28	2·31	0·336	—	—	—	26	2·30	0·197	—	—	—	—	—	—	—	—	—	—	—	—
9–11. viii.	14	2·22	0·263	—	—	—	15	2·21	0·179	—	—	—	—	—	—	—	—	—	—	—	—
31. viii.–2. ix.	15*	—	0·267	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11–13. ix.	9*	—	0·328	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3–5. x.	—	—	—	30	2·52	0·406	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9–11. x.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	28	2·05	0·136			
23–25. x.	—	—	—	25	2·68	0·451	—	—	—	—	—	—	—	—	—	22	2·17	0·217	20	2·36	0·274
30. x.–1. xi.	—	—	—	—	—	—	13	2·35	0·172	5	2·70	0·359	—	—	—	—	—	—	—	—	—
6–8. xi.	1	2·43	0·360	26	2·60	0·477	20	2·41	0·204	7	2·65	0·356	—	—	—	18	2·16	0·222	16	2·40	0·255
4–6. xii.	—	—	—	—	—	—	7	2·22	0·118	2	2·70	0·335	—	—	—	29	2·04	0·142	30	2·08	0·140
12–14. xii.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29	2·09	0·101	—	—	—
19–21. xii.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33	2·08	0·171	—	—	—
1957																					
9–11. i.	—	—	—	10	2·57	0·326	30	2·29	0·247	5	2·40	0·291	20	2·46	0·436	31	2·03	0·148	1	1·84	0·263
16–18. i.	—	—	—	—	—	—	29	2·35	0·332	12	2·47	0·415	28	2·51	0·412	25	2·07	0·188	23	2·00	0·338
17–19. i. Large	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19	2·31	0·209	—	—	—
17–19. i. Small	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18	1·99	0·151	—	—	—
30. i.–1. ii.	—	—	—	—	—	—	26	2·35	0·260	—	—	—	—	—	—	—	—	—	—	—	—
2–4. ii.	—	—	—	—	—	—	31†	2·42	0·200	—	—	—	—	—	—	—	—	—	—	—	—
12–14. ii.	28	2·44	0·316	22	2·57	0·305	7	2·54	0·261	1	2·21	0·193	8	2·54	0·259	4	2·27	0·153	1	2·21	0·279
12–14. iii.	32	2·42	0·347	28	2·52	0·383	11	2·40	0·238	—	—	—	—	—	—	—	—	—	—	—	—
2–4. iv. Large V	30	2·70	0·434	2	2·54	0·284	11	2·66	0·341	2	2·54	0·349	31	2·49	0·495	21	2·41	0·307	—	—	—
2–4. iv. Small V	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	30	2·23	0·276	—	—	—
18–20. iv. Deep	31	2·86	0·636	—	—	—	—	—	—	—	—	—	—	—	—	30	2·55	0·371	—	—	—
18–20. iv. Surface	30	2·74	0·736	—	—	—	—	—	—	—	—	—	—	—	—	21	2·49	0·387	—	—	—
23–25. iv. Deep	30	2·75	0·643	—	—	—	2	3·00	0·652	—	—	—	29	2·57	0·499	—	—	—	—	—	—
23–25. iv. Surface	30	2·72	0·569	15	2·75	0·454	—	—	—	—	—	—	8	2·56	0·453	—	—	—	—	—	—
10–12. v.	29	2·86	0·669	—	—	—	3	2·75	0·374	—	—	—	32	2·65	0·515	16	2·59	0·309	—	—	—
15–17. v.	10	2·82	0·591	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21–23. v.	31	2·76	0·632	—	—	—	10	2·74	0·337	—	—	—	31	2·66	0·456	22	2·46	0·364	—	—	—
4–6. vi.	28	2·67	0·566	—	—	—	—	—	—	—	—	—	15	2·66	0·377	24	2·51	0·327	—	—	—
6–10. vi.	10	2·73	0·545	—	—	—	—	—	—	—	—	—	—	—	—	12	2·67	0·310	—	—	—
6–10. vi.	—	2·75	0·578	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15–17. vi.	3	2·50	0·304	—	—	—	2	2·39	0·190	—	—	—	3	2·75	0·484	9	2·53	0·220	—	—	—
18–21. vi.	6	2·41	0·280	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9–11. vii.	27	2·35	0·391	—	—	—	2	2·59	0·246	—	—	—	8	2·24	0·380	18	2·27	0·239	—	—	—
16–18. vii.	31	2·31	0·304	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20–22. viii.	32	2·26	0·246	—	—	—	—	—	—	—	—	—	—	—	—	30	2·05	0·152	—	—	—
1–3. x.	30	2·35	0·268	—	—	—	—	—	—	—	—	—	—	—	—	30	2·17	0·182	—	—	—

* *C. finmarchicus* and *C. helgolandicus* not distinguished.

† At 7·0° C.

between 0·35 and 0·51 µl./*Calanus*/h and show little if any seasonal variation. The results for immature females resemble those for the ripe females before, but are decidedly lower during, the spring rise. At that time immature females were scarce and the results are based on only four experiments, each with only a few females. On an average, immature females use about 60% of the oxygen used by ripe females but unfortunately the highest value (23 April) is based on only two immature females, one of which was exceptionally large.

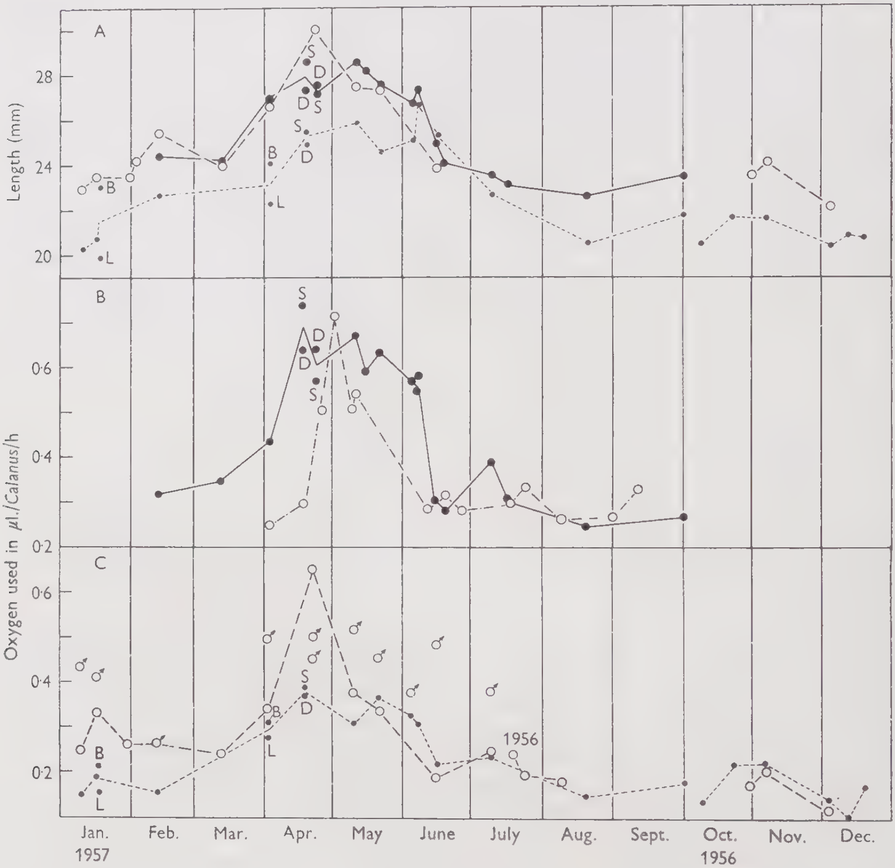

Fig. 1. Seasonal change in size and oxygen consumption. Continuous line, ripe female *Calanus finmarchicus*; dashed line, unripe female *C. finmarchicus*; dotted line, Stage V *C. finmarchicus*. (A) Length of carapace in mm. (B) O_2 consumption of ripe females; continuous line, 1957; dash-dot line, 1956. (C) O_2 consumption of unripe females, males and Stage V.

In Stage V (Fig. 1C) there is a slight increase in spring but, averaged over the high period, consumption is about 56% of that of ripe females, i.e. much the same as the immature.

When the results from all the experiments done with Dr A. G. Nicholls in 1930–32 were re-examined, it was found that as has already been suggested (Riley, Stommel & Bumpus, 1949; Gauld & Raymont, 1953), in them also there was a considerable seasonal variation. When those done at 10° C were arranged according to date, there was seen to be an irregular rise in the oxygen consumed from February to the end of April and several high values in the beginning and middle of May. The lowest values, those in February and March, were rather higher than those in 1956–57 and this might have been because of the method used.

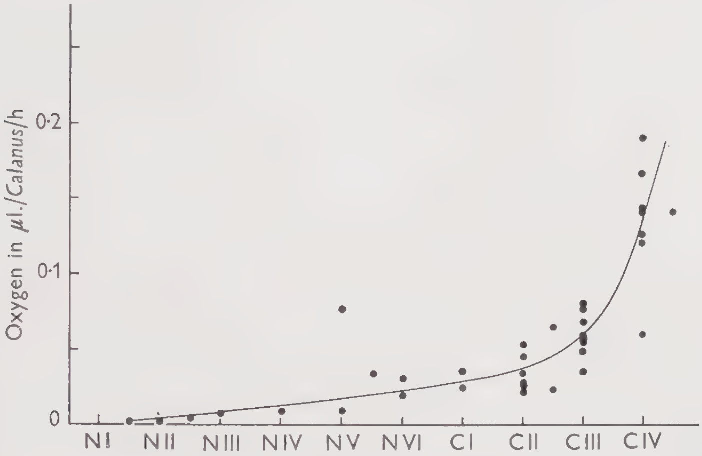

Fig. 2. Oxygen consumption of the early stages of *Calanus*. NI–NVI, nauplius stages; CI–CIV copepodite stages.

In the Clyde Sea Area the *helgolandicus* form is slightly larger than the *finmarchicus* and, as might be expected, the figures for respiration are slightly higher too. In spring 1957, however, when the two forms were almost the same size, the respiration of the ripe female *finmarchicus* was higher than that of the *helgolandicus* form. The *helgolandicus* form usually disappears before the first generation has developed and so there is no way of telling if they have a period of high respiration corresponding to that in *finmarchicus* (see, however, p. 467).

In both 1956 and 1957 *Calanus* were scarce during the summer and few early stages were obtainable. Their oxygen consumption was therefore measured mainly in April, May, and September to November. The experimental error of the determinations is relatively large and no clear seasonal cycle can be made out. The results are shown in Fig. 2 and Tables 3 and 4, and on the whole give a smooth curve ranging from 0·002 µl./*Calanus*/h for Nauplii I–II up to 0·15 µl./*Calanus*/h for Copepodite IV. There is no abrupt rise with the change from nauplius to copepodite.

There are several factors which might cause an increase in respiration during the spring months, and among these are increased light, increased food, increased temperature and increased size.

Light is a factor which has been found to have a marked effect in raising respiration in *Calanus*. It might therefore be expected that the first generation, which is often surface living, should have a higher respiration rate than other generations. On two occasions (see *D* and *S* on Fig. 1 B, C) samples of first-generation *Calanus* were taken from deep and surface water at the same time and their oxygen consumption compared. The results were contradictory, the surface catch having a higher respiration in one experiment and a slightly

TABLE 3. RESPIRATION OF COPEPODITES

1955 experiments all done at 0° C. 1956 experiments all done at 10° C. Times vary from 19 to 48 h.

	CI		CII		CIII		CIV	
Date	No.	µl./*C*/h	No.	µl./*C*/h	No.	µl./*C*/h	No.	µl./*C*/h
1955								
16–17. viii.	—	—	—	—	—	—	57	0·038
17–19. viii.	—	—	—	—	20	0·015	60	0·037
3–5. ix.	—	—	—	—	29	0·027	44	0·065
6–8. ix.	—	—	—	—	42	0·032	51	0·058
1956								
26–28. iii.	—	—	—	—	5	0·036		
24–26. iv.	—	—	—	—	2	0·049	3	0·141*
30. iv.–2. v.	—	—	—	—	—	—	3	0·167
7–8. v.	—	—	—	—	3	0·059	21	0·145
3–4. viii.	—			—	5	0·050	—	—
6–7. viii.	3	0·011	29	0·029	34	0·070	19	0·142
8–10. viii.	—	—	1	0·027	—	—	—	—
16–18. viii.	1	0·036	1	0·052	—	—	—	—
20–22. viii.	—	—	1	0·023	—	—	—	—
3–5. x.	—	—	—	—	6	0·057	—	—
9–11. x.	—	—	—	—	—	—	7	0·060
22–23. x.	—	—	3	0·054	7	0·081	10	0·127
29–30. x.	—	—	1	0·046	—	—	—	—
5–6. xi.	—	—	—	—	8	0·078	13	0·121

* Moulted to C V.

TABLE 4. RESPIRATION OF *CALANUS* NAUPLII

	Nauplii I–II		Nauplii II and II–III		Nauplius III		Nauplius IV		Nauplius V		Nauplius VI	
Date 1956	No.	µl./*C*/h	No.	µl./*C*/h	No.	µl./*C*/h	No.	µl./*C*/h	No.	µl./*C*/h	No.	µl./*C*/h
29–31. iii.	172	0·002	—	—	—	—	—	—	—	—	—	—
5–7. iv.	—	—	618	0·004	—	—	—	—	—	—	—	—
25–27. iv.	—	—	256	0·003	—	—	—	—	—	—	—	—
2–4. v.	548*	0·002	—	—	—	—	—	—	—	—	—	—
6–7. viii.	—	—	—	—	—	—	—	—	—	—	—	—
29–30. x.	—	—	—	—	—	—	—	—	1	0·010	—	—
5–6. xi.	—	—	—	—	7	0·008	2†	0·009	—	—	3	0·031
					21†	0·008	2†	0·032	1	0·080	1†	0·033

* Some Nauplii II–III. † One or more moults.

lower one in the other. Bainbridge (1952), from observations while diving, has described a continuous interchange between the *Calanus* at the surface and those deeper down so that a clear difference between them should not be expected. This, however, would not exclude the possibility that it was the greater exposure to light of the surface-living generation which caused the increased respiration.

In the English Channel it is not the *Calanus* of the first but those of a later generation in July or August which are found at the surface (Russell, 1928), although the form is *helgolandicus*. The respiration of those from the surface and from deeper water was measured in August 1957 at Plymouth, and was found to be no higher than that of the *finmarchicus* form in the Clyde Sea Area at the same time although it was the same size. We may therefore conclude that the higher light intensity received by those living near the surface is not responsible for the increase in respiration.

The increase in respiration in the spring of 1957 began at the same time as the diatom maximum, but this was over by 22 April, whereas respiration remained high until the beginning of June. It then dropped suddenly and remained low in spite of a second but smaller diatom increase in the first part of June.

Attempts to starve ripe female *Calanus* in the laboratory over a long period were not successful. A few specimens died and eggs were laid providing some food for the rest. A comparison of the respiration of these with that of fed *Calanus* showed little difference. Tests were also made with the filtrate from diatom culture to find out whether it increased the respiration of a variety of small copepods (*Centropages*, *Temora*, *Acartia*); there was no effect.

Two thyroid derivatives which might be expected to have an effect on respiration were tested at the suggestion of Dr J. M. Dodd, St Andrews. Sodium L-thyroxine and triiodothyronine, each in a dilution of 10^{-6}, were used, again with negative results.

The increase of temperature in spring is much too slight to be the sole cause of the rise in respiration (Marshall, Nicholls & Orr, 1935; Clarke & Bonnet, 1939) and in any case it goes on rising when the respiration falls in June and July.

In 1930–33 a series of experiments was made to find the effect of temperature. Each series was done with the same set of *Calanus*, which involved subjecting the same individuals to a series of rising temperatures up to 20° C and repeating the lower temperatures to see whether they had been damaged. Almost invariably the second test at the same temperature (usually 10° C) showed a lower result than the first indicating that the *Calanus* had suffered somewhat. The result chosen for publication was that in which this fall was least marked. All the experiments were done in February or August and, together with one done in August 1957 by the modified method, are shown in Fig. 3. It will be seen that there is a considerable range of variation and a tendency for the oxygen consumption to be higher at the same temperature in summer than in winter. The February females would be mainly unripe and the August ripe, which would account for the difference.

Another possible cause of the variation in respiration might be the variation in size. On two occasions Stage V *Calanus* were separated roughly by eye into a large and a small group (see *B* and *L* in Fig. 1 A, C) and the respiration measured separately. There was some overlap both in lengths and in the oxygen values in the two sets of bottles but the oxygen consumption of the large specimens was not so much greater as would be expected if size were the only cause of the respiration differences. In January, for instance, the average lengths were 2·31 and 1·99 mm and the oxygen consumption 0·21 and 0·15 μl./*Calanus*/h.

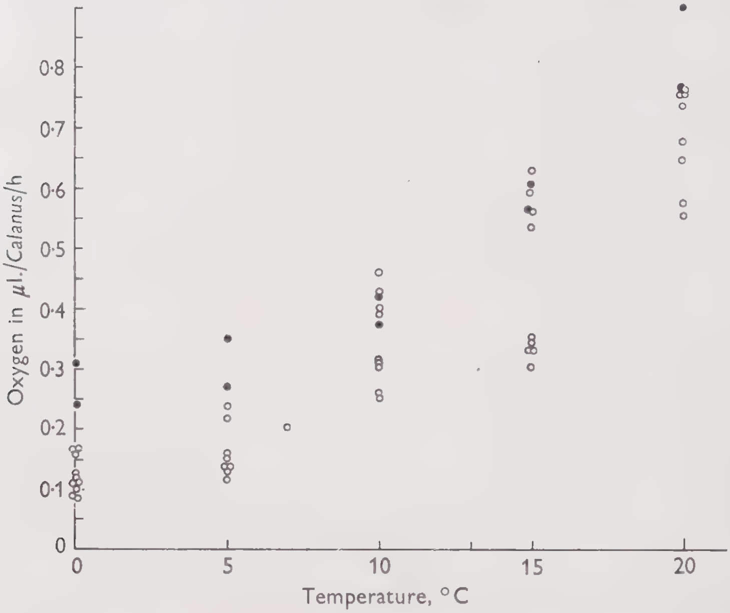

Fig. 3. Effect of temperature on the oxygen consumption of female *Calanus*. Open circles, February experiments; closed circles, August experiments.

The largest *Calanus* are those of the first generation of the year, appearing as adults about the beginning of April. The overwintering stock gradually dies off and at the end of April and in May only large first-generation *Calanus* are found. After this the length decreases gradually and irregularly until the winter when it is at its minimum. It is therefore the large females of the first generation which have the exceptionally high oxygen consumption. It is noteworthy, however, that the unripe females of the first generation, although comparable in size with the ripe, have a much lower respiration (Fig. 1 A, C).

Gauld & Raymont (1953) found differences in respiration between different generations of *Centropages* which did not depend on length.

If the length of the females is plotted against respiration (as can be done for some of the 1956 and all of the 1957 experiments), almost all the points for ripe *Calanus* lie well above those for unripe. The calculated lines (on a logarithmic scale) are shown in Fig. 4. The points for the ripe females of the first generation appear to be slightly above the upper line, which suggests that it is not length alone which accounts for their high respiration. This difference between the generations is not seen in unripe females.

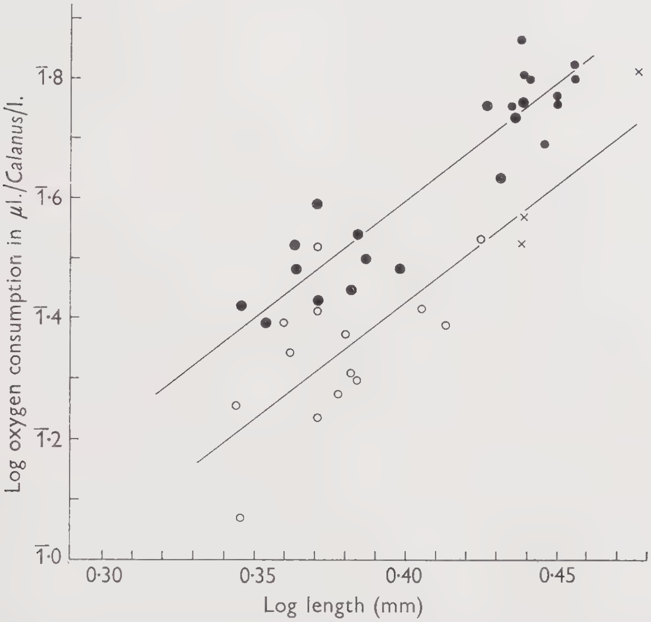

Fig. 4. Relation of length to oxygen consumption in *C. finmarchicus* females, 1956–57. Small closed circles, first-generation ripe females; large closed circles, all other ripe females; crosses, first-generation unripe females; open circles, all other unripe females. Lines calculated by the method of least squares.

The line for Stage V *Calanus* is not shown but lies between those for the ripe and the unripe. Not enough data are available for either males or for the *helgolandicus* form, although the relationship is not widely different.

A more useful measure than size might be the oxygen consumed per mg of *Calanus*. Unfortunately it is not possible to plot the respiration of the experimental animals against their own weight, but a series of weekly measurements of length and dry weight of male, female and Stage V *Calanus* is available from Loch Striven in 1933 (Marshall, Nicholls & Orr, 1934) and these may fairly be used.

When weight is plotted against length for the 1933 *Calanus* females, they fall into several distinct groups (Fig. 5). The points for the winter females are scattered and lie mainly on the lower part of the graph. These *Calanus* are the survivors of the late summer broods and the lower values show their loss of weight during the winter. Those of the first generation are, for a given length, lighter than those of the second and third. Thus although they have the maximum weight for the year, it is only because of their greater length.

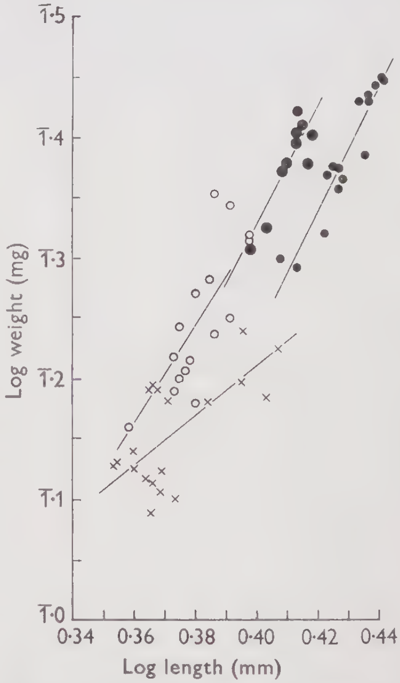

Fig. 5. Relation of weight to length in *Calanus* females from Loch Striven, 1933. Crosses, winter; small closed circles, first generation; large closed circles, second generation; open circles, third and later generations. Lines calculated by the method of least squares.

When from the length in 1956–57, the weight of the ripe females is read off in Fig. 5, and the respiration per mg *Calanus* calculated for the points in Fig. 1B, they lie irregularly and are only slightly higher during the spring than later in the year.

A factor which should be taken into account is the state of maturity of the females. The 1933 weights represent an unclassified sample of all females, whereas the respiration experiments were done on either ripe or immature, and, since the length–respiration lines for these differ considerably, the length–weight lines might be expected to do the same. The weights of the 1956–57 ripe females, as read from the line in Fig. 5, will be too low and the respiration thus appears higher for the length than one would expect. The converse holds for the unripe females. The exceptionally high oxygen consumption of the ripe females of the first generation may be partly caused by the extra weight of the eggs.

If the relationship of length to dry weight for the whole populations of males, females and Stage V *Calanus* in 1933 is calculated, the lines for males and females are not significantly different. Stage V is, however, much heavier per unit length and its line is significantly different from the others. In spite of this the respiration of the Stage V is lower.

The figures for Stage V can be analysed into broods as for the females in Fig. 5. They then fall into the same groups; a small and light overwintering generation, a large but relatively light first generation and a succession of smaller but relatively heavier broods in summer. The overwintering Stage V show a fall in weight from autumn to January. If the respiration per mg dry weight is calculated from the 1933 figures, the seasonal changes are, as for females, very irregular but show a tendency to be lower for the first than for the overwintering generation. This is probably because of the much higher fat content of the former.

We should like to thank the Trustees of the Browne Fund for a grant which enabled us to work on *Calanus* in the English Channel. We are also grateful to the Director and Staff of the Plymouth Laboratory for their hospitality. Mr T. B. Bagenal gave helpful advice on statistical problems and Dr J. M. Dodd supplied us with thyroxine compounds; to them and to the Master and crew of the research vessels '*Calanus*' and '*Mizpah*' who kept us supplied with tow-nettings we should like to express our thanks.

SUMMARY

The seasonal changes in the respiration of *Calanus* are considerable and are, on the whole, related to size and therefore to weight. Length alone is not enough to account for the differences since ripe females, although the same length as unripe, have a markedly higher respiration. In addition, by taking samples of large and of small *Calanus* of a single stage, it was shown that the difference in respiration was small. Neither is weight by itself enough to account for the difference between groups. Stage V *Calanus* are, for a given length, heavier even than ripe females and yet their oxygen utilization is low. In this instance, however, an important part of the weight consists of fat which is a food reserve and not actively metabolizing. The difference of weight between ripe and unripe females is not known, but ripe females must be heavier and this will account for their higher oxygen consumption.

Although the oxygen and therefore the food required during the spring months is high, at that time the phytoplankton is at its maximum and is probably sufficient to fulfil all needs. Egg-laying depends on the food supply and it is then that *Calanus* starts breeding. In winter, on the other hand, the *Calanus* is present as Stage V and oxygen consumption is little more than half what earlier figures suggested. No 'hibernation' seems to take place but the population is living in an economical way for Stage V use little oxygen, live in deep water and do not undertake diurnal vertical migration.

At 10° C ripe female *Calanus* will require daily from 3·9–7·2% of their body weight as dry matter in summer and from 2·8–6·7% in winter. Stage V will require 2·3–3·1% in summer and 1·4–3·3% in winter. The higher values are for carbohydrate and the lower for fat.

It is difficult to believe that *Calanus* in winter will be able to find enough food by filtration alone. The fact that in the winter months it depends more on predation may account for its survival.

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UNDERWATER OBSERVATIONS ON THE FAUNA OF SHALLOW ROCKY AREAS IN THE NEIGHBOURHOOD OF PLYMOUTH

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(Text-fig. 1)

Since 1954 I have made many underwater explorations in the neighbourhood of Plymouth using aqualung equipment. Diving has been generally restricted to rocky areas within the 15-fathom line. In these areas, because of the difficulty of dredging, there has been little information available concerning the general pattern of the fauna; as Drach (1952) has pointed out, direct study with diving apparatus can not only give data on the depths at which various species flourish, but also the opportunity to collect the more secluded animals. It has not been possible to obtain such detailed results as Kitching, Macan & Gilson (1934), or Knight-Jones & W. C. Jones (1955), but these notes may provide an outline of the fauna as observed underwater in relation to the bottom topography.

To try to obtain as simple a picture of the fauna as possible, attention has been concentrated on places where the sessile animals are not intermixed with algae. In the shallowest water, therefore, the localities described are those with rock facies sufficiently shaded to exclude all but occasional algal plants.

Nearly all the best diving grounds are fully exposed to south-westerly winds; as these winds are generally prevalent it is frequently only possible to obtain sufficiently calm sea conditions once to twice a week even during the summer months. It is therefore very difficult to adhere to any fixed programme, and unless one is fortunate as I have been in having my own boat available at any time to take advantage of calm spells, opportunities for diving will be very restricted.

1. THE WRECK, WHITSAND BAY

The sunken hull of a 'Liberty' ship lies on the sand in 9 fm. (16 m) about $\frac{3}{4}$ mile offshore, towards the eastern end of Whitsand Bay. The one remaining mast, which is always uncovered, forms a convenient mark and mooring post.

On the remains of the top deck and superstructure, which are about 15 ft. (4·5 m) deep, there is some algal vegetation, including *Alaria* and rather scattered *Laminaria hyperborea* and *Saccorhiza* plants. Large numbers of small mussels (*Mytilus* sp.) occur around the top deck and, associated with them, numerous small *Asterias rubens*. Curiously enough, only small starfish were seen both in 1955 and 1957, although the supply of mussels appeared amply sufficient to produce rapid growth. Possibly the *Asterias* population is considerably reduced by severe winter gales.

The two sides of the hull are both well colonized. On the west side in 1955 there were numerous *Ophiothrix fragilis*, various encrusting ascidians, especially *Botrylloides leachi*, sponges—particularly *Suberites domuncula* (= *Ficulina ficus* (L.))—and an abundance of *Metridium dianthus*. The *Metridium* flourish especially on the underside of any horizontal surfaces, such as occur at the openings on to the 'tweendecks. This is the only inshore locality outside the Sound where many *Metridium* have been found, showing that it is lack of a suitable habitat rather than inability to withstand wave action which normally restricts them to pier piles and dock walls.

2. QUEENER POINT

Queener Point is situated ½ mile north of Rame Head at the east end of Whitsand Bay. From the point an area of submerged rocks runs out for more than ¼ mile. Except for Peader Rock, which dries at L.W.O.S.T.L., the depth varies from 4 to 7 fm. (7–13 m). Although the rock is the usual Dartmouth slate the strata run in the vertical plane and consequently the rock has been eroded in an unusual pattern—this area being notable for the large number of shallow gullies running roughly E.–W. in direction. These gullies are very roughly 6–8 ft. (2–2½ m) deep and 8–14 ft. (2·4–4·2 m) wide; their side walls provide a much more extensive area of vertical rock surface than is generally present on this part of the coast. In the narrower gullies the *Laminaria* vegetation is greatly reduced or absent, permitting the development of a moderately rich sessile fauna. It is not possible to do more than point out some of the commonest or most conspicuous species. Groups of the small orange ascidian *Stolonica socialis* are very common on the upper parts of the gully walls and in other situations where the *Laminaria* is not dense. Many of the small white anemone *Actinothoë sphyrodeta* also occur frequently in similar places. On the vertical rock walls there is a variety of sponges, ascidians and encrusting Bryozoa, together with scattered red algae especially *Delesseria sanguinea* which becomes covered, by the autumn, with a great abundance of the tiny white foraminiferan *Haliphysema tumanowiczi*. The case of this species is composed of sponge spicules. The encrusting sponges *Amphilectus fucorum* and *Hemimycale columella* are common, while tufts of the rare reddish horny sponge *Ulosa tupha* have been taken on one or two occasions. Colonies of *Alcyonium digitatum* are fairly common but not evenly distributed throughout the area, although no special habitat preferences were apparent. On the more sheltered or overhanging rock walls there are many cup corals, *Caryophyllia smithi*, some with the barnacle *Pyrgoma anglicum* growing on them. Also preferring sheltered conditions, colonies of *Bugula turbinatum* and other erect Bryozoa are fairly common. The finely branched black tentacles of *Cucumaria normani* are frequently seen protruding from holes in the gully walls; similarly, one may find the large tubicolous polychaete worm *Bispira volutacornis* made conspicuous by its double whorl of branchial filaments. Some specimens of the beautiful red serpulid *Protula tubularia* have been seen, but since the opening of its calcareous tube is invariably set well back in a rock crevice this species is extremely difficult to collect.

In early summer the ascidian *Archidistoma aggregatum* is quite common but disappears by the end of July.

3. THE DRAYSTONE LEDGE

One dive was made about 20 yd. west of the Draystone Ledge Buoy off Penlee Point, depth about 6 fm. (11 m). The sea bed consists of low-lying reefs of slate not more than 2 or 3 ft. high and interspersed with patches of coarse gravel. Many *Holothuria forskali* were present, together with *Stolonica socialis* and red algae probably largely *Heterosiphonia* sp.

4. INNER PENLEE POINT

(Admiralty Chart no. 30, as Inner Broady Cove)

This is the only satisfactory position outside the breakwater where diving is possible with south-westerly winds. The rocks run out to depths of 4 or 5 fm. (7 or 9 m), sometimes as reefs with sand between or as a gently shelving slope fringed by the sand. Generally the rocks are thickly covered with *Laminaria* even though the water is usually muddy and turbid since the main tidal flow in and out of the Sound runs close by. In the sand adjoining the rocks there are sometimes numerous *Arenicola* castings but they do not appear to extend beyond depths of 4–5 fm. No sublittoral records of *Arenicola* appear in the 1957 Fauna List. It is, however, unlikely that the species generally penetrates more than a short distance below the lowest tidal level in the neighbourhood.

After the first few dives, this area was thought to have only a poor sessile fauna, limited to scattered pot-holes and overhanging ledges. The sponges *Tethya aurantium*, *Stelligera stuposa* and *Raspailia hispida* were found in these places together with a variety of sessile animals though without any species being noticeably common.

In 1957, however, an interesting gully with an extensive overhanging rock wall was found off the south side of Inner Broady Cove. The approximate dimensions and depths in this gully are shown in the sketch chart (Fig. 1). In a side branch from this gully large numbers of *Phoronis hippocrepia* live in a belt of dead *Pomatoceros* tubes about 1–2 ft. above the sand. The end of the side branch terminates in an overhanging wall about 4 ft. high; this provides a sheltered rock surface colonized by many *Caryophyllia smithi* together with a few *Balanophyllia regia*, Gosse's golden cup coral. In the main gully the south wall, which varies in height from 6 to 10 ft., and is undercut in many places, has the better developed sessile fauna. Where the overhang is considerable there is a rich growth of the brightly coloured 'anemone' *Corynactis viridis*. *Stolonica socialis* is again common on the upper parts of the wall near

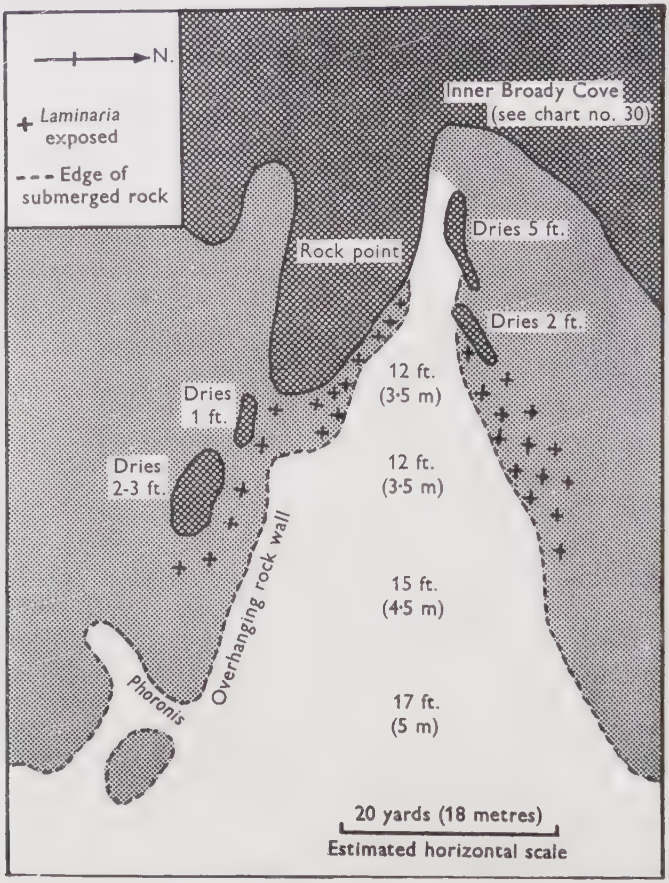
N.
+ *Laminaria* exposed
--- Edge of submerged rock
Inner Broady Cove (see chart no. 30)
Rock point
Dries 5 ft.
Dries 2 ft.
12 ft. (3·5 m)
Dries 1 ft.
Dries 2-3 ft.
12 ft. (3·5 m)
Overhanging rock wall
15 ft. (4·5 m)
Phoronis
17 ft. (5 m)
20 yards (18 metres)
Estimated horizontal scale

Fig. 1. Sketch plan of gully about ¼ mile north of Penlee Point. Rocks exposed at low tide shown by dark tint, submerged rock by light tint: sandy bottom untinted.

the *Laminaria* fringe. Other parts of the vertical rock surface bear a variety of encrusting animals, the bulk of which are sponges and ascidians; these have not yet been sampled as it is hoped that an intensive survey will be made sometime in the future.

5. THE TINKER SHOAL

This is a large shoal area south of the main breakwater. The bottom is slate rock often in the form of low reefs with occasional stony or gravel patches. No steep-walled gullies have yet been found but there are some ledges with vertical rock walls of up to a height of six feet. A fine deposit of mud is generally present in the sheltered crevices of these ledges. The fauna is rather sparse, *Stolonica socialis* again being common together with the sponge *Myxilla rosacea*.

6. THE SLIMERS

A small isolated half tide reef at the west end of Wembury Bay, the Inner Slimers, lies about $\frac{1}{4}$ mile east of the Mewstone. On the north or landward side of the reef there is a projecting rise of rock just below L.W.O.S.T.L. which protects and shades the rock surface below it. The erect forms of Bryozoa (*Crisia* spp., and *Scrupocellaria reptans*) are particularly common in this sheltered niche. There are also various sponges forming a thin loose mat held between the stalks of the Bryozoan colonies; *Suberites epiphytum*, *Myxilla rosacea* and *Dysidea fragilis* are all common together with the firmer white crust of *Leuconia nivea*. From a rock sample of 501 cm^2, 131 small amphipods (chiefly *Parajassa*) were counted.

7. KITCHING'S GULLY, WEMBURY BAY

This gully lies 60 yd. east of the Tomb Rock shown on Admiralty Chart no. 95. The sublittoral fauna was surveyed in 1931 and 1932 by Kitching *et al.* (1934). It has been interesting to look for changes in the fauna which have taken place in the 25 years since this survey was made. The sponge *Halichondria panicea* is no longer common and there is no sign of a *Distomus–Halichondria* association. The only *Halichondria* found during 1956 and 1957 were a few patches at the shallow end of the gully remote from the area of the survey. The *Distomus variolosus* is still abundant, forming bright reddish patches through which the large siphons of *Polycarpa pomaria* may frequently be seen projecting. The large majority of the *Distomus* grow on the tough leathery tests of the *Polycarpa* which curiously enough was not taken by Kitching. *Corynactis* is rather more common now than would appear from their survey, particularly on the shaded area such as Kitching's position M p. 683, previously dominated by sponges. The south patch of *Stolonica socialis* still exists at the seaward end of the gully.

On the west side of the small rocky point, which forms the west side of Kitching's gully, there is a wide undercut ledge. On the underside of this ledge numerous small orange *Sagartia* sp. (probably *Sagartia elegans*) have been abundant during the last two summers. *Distomus* is still common but not so widespread as in the gully.

On the east side of the gully a small area of submerged rocks provides rather similar conditions; there are still small patches of *Distomus*, but in all shaded areas *Corynactis* is more abundant. Two hydroids, *Plumularia setacea* and *Sertularia operculata* are also very common in places.

8. THE STOKE POINT AREA

The fauna of a rock gully near Stoke Point Rock has already been described (Forster, 1954). On the latest copies of Admiralty Chart no. 1267 Stoke Point Rock has been renamed Hilsea Point Rock following the Ordnance Survey, but as Stoke Point is the name in general use it seems better to retain it for the present.

On the part of the shore lying about north-east from Stoke Point Rock several long narrow reefs run out in a south-westerly direction. Most of the gullies between these reefs have a purely algal covering, however one rather narrower gully was found with side walls at least 15 ft. high and an abundant sessile fauna. The position of this gully is 037° T from Stoke Point Rock, distance $3\frac{1}{2}$ cables. The depth at the bottom is about 4 fm. (7 m). The west side wall has a slight overhang; on it occur several species usually only found in much deeper water; there was one colony of the gorgonian *Eunicella verrucosa* and several of the ascidians *Phallusia mammillata* and *Diazona violacea*. There are numerous *Caryophyllia*, the feathery bryozoan *Crisia* spp. in abundance, together with the following sponges: *Dercitus bucklandi* in crevices, *Hemimycale columella*, *Pachymatisma johnstonia*, *Mycale macilenta*, *Leucosolenia coriacea* and one small patch of orange incrustation identified as *Antho involvens*.

About 600 yd. farther east along the coast, the three large Netton Rocks and submerged reef form a slight breakwater which makes it possible to anchor a boat close inshore. There are a number of gullies in this area (named 'Bloody Cove' on the $2\frac{1}{2}$ in. Ordnance Survey maps) roughly similar in proportion to those of Queener Point and Inner Broady Cove but with scarcely any sessile fauna. Instead the vertical rock walls are covered with algae in spite of the shading caused by overhanging *Laminaria* plants. The algae are often species more generally found in deeper water, e.g. *Dictyopteris membranacea* and *Halopteris filicina*. This increase in algal coverage is doubtless due to the lack of suspended material in the water, which is always much clearer than it is nearer the Sound. Secchi disk readings were twice taken at Inner Broady Cove and then as quickly as possible off Netton Rocks, the results were:

Inner Broady Cove	$6–6\frac{1}{4}$ m
Netton Rocks	11–12 m

A few narrow gullies not more than 3 ft wide did contain some sessile animals, encrusting Bryozoa and ascidians, but the algal coverage was still estimated to be over 50%.

9. SALCOMBE

Several deep clefts have been found in the submerged reef which runs for a short distance eastwards from the Great Mewstone off Bolt Head. These clefts have vertical walls over 20 ft. in depth with a rich sessile fauna. Although only one short dive has been made in this position, the ascidian *Distomus* was found to be clearly abundant. This is of interest as being the only position other than Kitching's gully in Wembury Bay where this ascidian is really abundant. *Corynactis* and *Crisia* spp. are also fairly common.

10. THE EDDYSTONE ROCKS

The Admiralty Chart no. 1267 shows a large-scale plan of the Eddystone rocks, which lie about 9 miles offshore. Several rocks are uncovered at low tide, the Southern Reef in particular forming a small natural breakwater on which landing is occasionally possible in extremely calm conditions. The area of reef within the 10 fm. line is a little over $\frac{1}{10}$ sq.mile. The water is generally much clearer than at any of the inshore positions except Bloody Cove. The rock is a very hard gneiss.

Several dives have been made on a line running about south-west from the mid-point of the south reef. From L.W.O.S.T.L. there is a narrow fringe of the brown alga *Alaria*, which is quickly replaced by large *Laminaria hyperborea* plants. Many brown tubes of the amphipod *Parajassa pelagica* are common in the space between the *Laminaria* holdfasts to a depth of about 7 fm. (13 m), penetrating much farther than under inshore conditions; their limit at Wembury being only 1–2 fm. (2–4 m). The lower limit of the *Laminaria* has not yet been ascertained, below 12 fm. (22 m), however, it becomes sparse. The reef slopes fairly steeply down to 10–12 fm. (18–22 m), although most of the surface is algal covered there are some ridges, clefts, and occasional very large boulders, with vertical or overhanging sides. Wherever the rock is sufficiently shaded *Corynactis* is particularly common, the different colour varieties forming red, green or brown patches. The light green variety appeared bright yellow at 4 fm. (7 m). In well-sheltered crevices from 7–8 fm. (13–15 m) downwards, besides *Corynactis*, towards the outside there is generally a rich growth of *Crisia* spp. giving the effect of a white moss. At slightly greater depths—around 11 fm. (20 m) the *Crisia* becomes more extensive. From 13 fm. (24 m) downwards the slope becomes gradual and these rocks are interspersed with patches of coarse shell gravel.

At all depths it is difficult to collect small stones or boulders as they are invariably very tightly packed. The few stones which have been examined have many *Caryophyllia smithi*, together with thin yellow encrusting sponges; notably *Spanioplon armaturum*, *Myxilla rosacea* and *Hymedesmia* spp.

On the east side of the Southern Reef it is possible to anchor within a few yards of the rock if there is a westerly wind and easterly going tide. There are several rocks which appear dangerous in the clear water, but actually have 2–3 fm. (4–6 m) of water over them at L.W.O.S.T. These rocks form one side of a gully with a maximum depth of 9 fm. (16 m). Even the nearly vertical sides of the gully bear some *Laminaria* plants, but in a few overhanging places there are again fine *Corynactis* growths, and also numerous *Actinothoë sphyrodeta*; this species reaches a much greater individual size (disk diameter of nearly 1 in.) than it does inshore.

On the floor of the gully *Laminaria* plants are rather infrequent, being largely replaced by *Dictyota dichotoma* and *Heterosiphonia*. Several fine pinkish colonies of the ascidian *Clavelina* sp. have been observed, together with purple clumps of the sponge *Adocia cinerea* and the ubiquitous *Hemimycale columella*.

150 *yards north of lighthouse*

The depth at this position is a little over 10 fm. (18 m), and the bottom consists of large boulders and stones overgrown by tall *Laminaria* plants with here and there even larger plants of *Saccorhiza bulbosa*. The *Laminaria* stipes are well colonized by various red algae and various animals, chiefly hydroids and Bryozoa; *Sertularia operculata* is particularly common. *Echinus esculentus* is also abundant and has an estimated density of one sea urchin to about 5–10 m^2. In this area the *Echinus* are frequently observed climbing up the *Laminaria* stipes.

60 *yards north-west from old lighthouse stump*

The reef slopes rather steeply down from 5 to 12 fm. (9–22 m). There are again fine growths of *Corynactis* on several overhanging rock faces. Two specimens of *Lima hians*, a tunnel-building lamellibranch, were taken from underneath small boulders in this area.

II. THE HAND DEEPS

A misnamed submerged reef, the Hand Deeps, with a minimum depth of 4 fm. (7 m) lies 3½ miles north-west from the Eddystone. The reef is composed of a micaceous schist evidently not so resistant to the waves as the Eddystone rock. In one position on the south side of the reef at 13 fm. (24 m) the rock slopes gently downwards and the bottom is largely strewn with boulders, the largest being 4 or 5 ft. in height. *Laminaria* was still present at this depth. Two dives have been made on the north side of the reef.

In the first dive a steeply sloping rock bottom was found with a gradient of about 1 in 2 from 9 fm. (16 m) downwards. There were no *Laminaria* plants after 12 fm. (22 m) and no other algae except *Lithothamnion* after 14 fm. (27 m). From 12 to 17 fm. (22–31 m) *Corynactis* was again abundant on vertical surfaces, while *Caryophyllia* and *Alcyonium digitatum* were common. There were also scattered hydroid colonies *Nemertesia* sp. in evidence. The second dive was made in a position probably near the north-east corner of the reef. At 12½ fm. (23 m) there were scattered *Laminaria* plants growing on a gentle rock slope, these were replaced completely at 15 fm. (27 m) by *Dictyopteris* and various red algae. Numerous *Echinus esculentus* were also present.

12. THE EAST RUTTS AREA

One dive has been made on a rock pinnacle, depth 7 fm. (13 m), lying about 5 miles offshore in Bigbury Bay. This pinnacle is situated 1 mile to the east of the East Rutts. The fauna and flora at 8–9 fm. (14–16 m) depth are generally similar to those of Stoke Point Rock. There are large yellow clumps of the sponge *Cliona celata*, colonies of *Alcyonium digitatum* and numerous *Echinus* and *Holothuria*. In a small pit two fine colonies of the ascidian *Diazona violacea* were observed. This area is noteworthy for the presence of many clumps of the sponge *Halichondria panicea* in the unusual form shown in Bowerbank, Vol. 3, plate xl, figs. 2 and 5. Elsewhere in the Plymouth area *Halichondria* has not been found below about 2 fm. (4 m). Whether its abundance here can be related to the different rock substratum is not certain, but there can be little difference in the physical conditions compared with Stoke Point other than the nature of the rock.

DISCUSSION

In general the sessile fauna of submerged rocks off Plymouth tends to be rich in sponges, polyzoans and ascidians: compared with the low-tide area it is rather poor in actinians, and there are few gastropods apart from nudibranchs. The only common cirripede, *Verruca stroemia*, is mostly restricted to the underside of stones. Apart from *Corynactis* on the outlying reefs, no single species has been observed which could be regarded as dominant over an appreciable area. This situation contrasts with Bardsey Island, where Knight-Jones & W. C. Jones (1955) found various species of Polyzoa to be dominant, and differs also from Barn Pool in Plymouth Sound where the ascidian *Dendrodoa grossularia* covers all the stones at depths of 2–4 fm. (4–7 m) and forms a belt just below the *Laminaria*.

On the outlying reefs it would be reasonable to regard *Corynactis* as a dominant species on overhanging or shaded rock surfaces; but closer inshore, though still very common, it is generally mixed with other species, and for no obvious reason is virtually absent from the Queener Point area.

The general impression of the sessile fauna, particularly below 5 fm. (9 m), as it might be observed on a gully wall 3 or 4 m high is of a random dispersal of most species, with encrusting forms rarely managing to cover an area of $\frac{1}{20}$ m^2. A possible explanation of this effect is the browsing habit of the *Echinus esculentus*. A few *Echinus* will keep walls of an aquarium tank, 2–3 m^2 in area, almost completely free from sessile animals; since in most localities they are common below 8 fm. (15 m) the *Echinus* can be assumed to be continually sweeping clear small areas of rock throughout the course of the summer and to some extent throughout the year. The places available for larval settlement at any one time would therefore be widely scattered. Predation of the youngest stages may be expected from decapod crustacea and asteroids; which would again tend to prevent the growth of any large group of a single species.

As Lilly, Sloane, Bassindale, Ebling & Kitching (1953) observe, many observations and experiments on small areas will be necessary before the effects of predation can be assessed.

Part of the diving gear was made available by a grant from the Royal Society. I am grateful to many people who have acted as diver's attendant and always managed to recover me safely.

SUMMARY

A brief description is given of the commonest sessile animals observed by diving from twelve positions near Plymouth, including three offshore reefs. The coelenterate *Corynactis viridis* is generally abundant on shaded rock surfaces. Many sessile species, even where common, tend to be dispersed in scattered patches or colonies; from this it is suggested that their distribution is affected by predation from browsing animals, particularly *Echinus esculentus*.

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APPENDIX

The numbers of dives made at the localities which have been described are tabulated. The period is from 1954 to 1957. The numbers include dives made specifically for collecting purposes when faunistic observation is very limited. The time spent underwater has not always been recorded, but shallow dives generally last at least 30 min, and for deeper areas the average would be about 20 min.

Area ...	1	2	3	4	5	6	7	8	9	10	11	12
No. of dives ...	3	18	1	11	3	3	6	6	1	9	3	1

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THE SPREAD OF *ELMINIUS MODESTUS* DARWIN IN NORTH-WEST EUROPE

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(Text-figs. 1–9)

The presence in northern waters of the Australasian barnacle *Elminius modestus* was first noted by Bishop (1947). In the course of examining some bakelite plates, which had been exposed to fouling in Chichester Harbour, he found a settlement of barnacles which differed from all indigenous species in having only four instead of six compartments. They agreed in every respect with Darwin's description of *E. modestus*. The settlement of many spat on this one plate indicated that the species was well established at least in Chichester Harbour. Crisp & Chipperfield (1948) stated that the species was present in 1947 along the whole of the south-east of England, that it had been present in the River Crouch since 1945, and had appeared also in South Wales. They also commented that the habitats favoured by the species in Britain were similar to those which it occupied in New Zealand (Moore, 1944). Knight-Jones (1948) reported that the prevalence of *Elminius* in the River Crouch constituted a further threat to the Essex oyster beds; he also recorded it from the Helford River in Cornwall. Fig. 1 shows the known distribution at the end of 1947, and at the time of writing (1956).

It is clear that the species must have occurred in Britain for several seasons prior to 1945 when it was present already at two widely separated places, viz. Chichester Harbour and the River Crouch. Stubbings (1950) has confirmed its prior occurrence in the Portsmouth area, based on examination of material collected before 1945, but it is not possible to give any precise information as to the year of its arrival there.

The earliest stages of colonization have therefore escaped notice, perhaps inevitably in view of the closing of the beaches of southern England during the war. It seems not improbable that the establishment of the species had some connexion with unusual conditions at the outbreak of war, in so far as they affected shipping from Australasia. It is, moreover, fairly certain that *E. modestus* was not prevalent at this time in many areas where it was very abundant in 1946.

Table 1 brings together several pieces of evidence to this effect, all based on the examination of material collected prior to 1940. This material consisted chiefly of shells and other substrata collected both from shallow dredge hauls

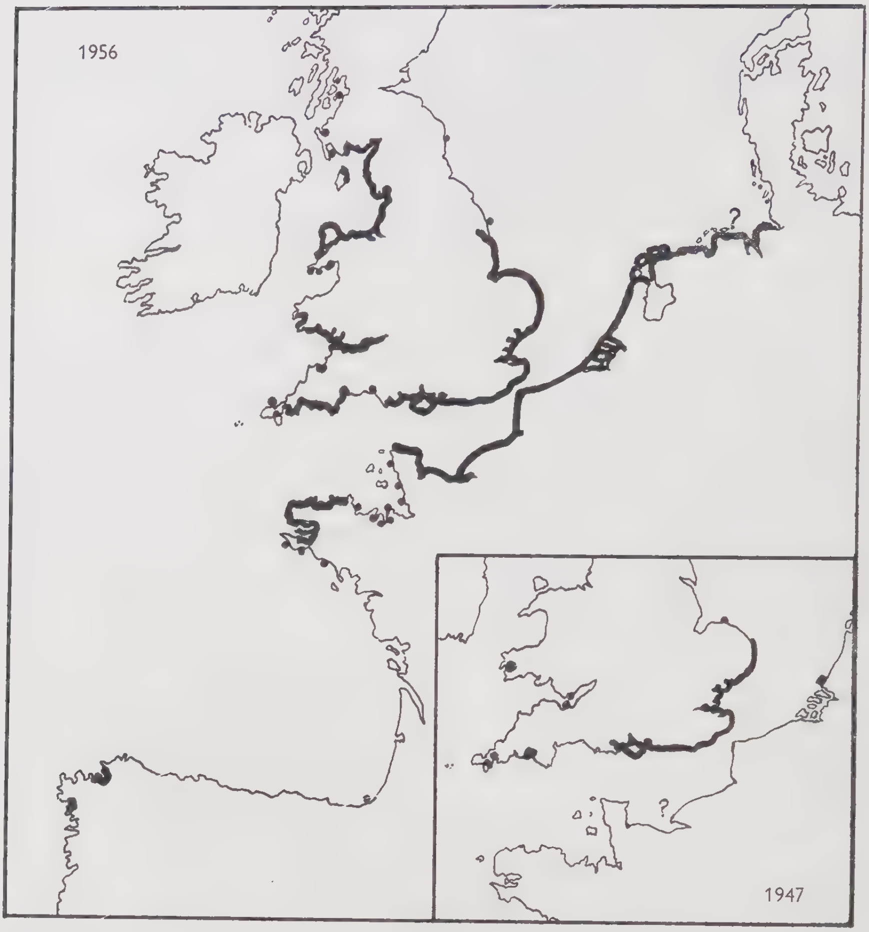

Fig. 1. Parts of the coast of Europe on which *Elminius modestus* was known to have been present in 1956, shown by a thickening of the coastline. Inset, same for 1947.

TABLE 1. OBSERVATIONS ON BARNACLE SETTLEMENT PRIOR TO 1940

Source of material	No. of barnacles found
River Alde, 1931. Shells and stones from low-water mark	*Balanus balanoides* 111 *B. crenatus* and *improvisus* 86 *B. porcatus* 1
Whitstable, 1937. Dredged shells	*B. improvisus* 1500
Tollesbury, 1939. Scrapings from piles	*B. balanoides* 33 *B. improvisus* 4
Steeple stone, River Blackwater, 1938. Scrapings from stones near low-water mark	*B. balanoides* 252 *B. improvisus* 2
Southend, 1938. Plankton hauls	*B. balanoides*, *B. crenatus* and *B. improvisus* larvae only

and from the shore, but also included a series of plankton samples taken from the Thames estuary at Southend throughout 1938 (Wells, 1938). In the plankton samples taken in the early part of the year, numerous larvae of *Balanus balanoides* and *B. crenatus* were identified, while in those taken during summer *B. improvisus* larvae were abundant (Jones & Crisp, 1954), but no *Elminius modestus* larvae were found in any of them.

Since 1946 a careful watch has been kept on the distribution of *E. modestus* in Britain. Quantitative records of its population density have been made repeatedly at numerous easily accessible stations along the coast of Great Britain, while visits have been made to those parts of the coast from which it has hitherto been absent as frequently as time and opportunity have permitted, in order to check its spread into new areas.

Changes are still going on. However, it seems unlikely that further changes will materially alter the general principles which follow from observations made up to the end of 1955. Nor is it likely that any new records will now be discovered to throw light on the early history of its arrival in British waters. It is therefore opportune to describe such changes as have been observed up to the present time.

The work reported in this paper was partly carried out during the period of my employment by I.C.I. Ltd. Paints Division, to whom I am indebted for access to my notes and records. I am also indebted to many colleagues who have from time to time given me information about the species, and whose records appear in the detailed appendix at the end of this paper. The work also constituted a part of a general investigation of British shores, for which generous grants were received from the Browne Fund of the Royal Society.

CHANGES IN DISTRIBUTION SINCE 1946

SOUTH-EAST ENGLAND

Records of the distribution of *Elminius* between the Isle of Wight and the Thames indicate no major change since 1946, as the species was already well established in the area at that time. Nevertheless, there is evidence that between 1946 and 1950 it was increasing steadily in abundance. This is demonstrated in Table 2A which gives the annual spatfall of *Elminius* during this period based on counts of numbers settling on regularly changed surfaces. The figure for 1946 is not entirely comparable with the others, since it was taken in the Blackwater, at West Mersea, a few miles to the north of the River Crouch where the rest of the observations were made. The Blackwater is probably not a more suitable river for *Elminius* than the Crouch, yet the records for 1946 in the Blackwater indicated a heavier spatfall than those for 1947 in the Crouch. This suggests that the severe winter of 1946–47 had an adverse effect. The lack of correspondence between the settlement on continuously submerged panels on a raft and on plates exposed between the tide-marks, especially between 1949 and 1950, is difficult to account for. The intertidal exposures probably give the best comparisons year by year, as they were made at the same place and in the vicinity of previously settled individuals, which are known to encourage settlement (Knight-Jones & Stevenson, 1950; Knight-Jones & Crisp, 1953; Knight-Jones, 1953). The settlement on plates exposed on the raft may have varied with its location in the tideway (it was shifted slightly from time to time) and with the amount of settlement already on it. Indeed, the spatfall in different parts of the river may show considerable variation, as demonstrated by Knight-Jones (1952). His observations from 1947–49 are given in Table 2B. They have been modified from the form given in his paper to make them directly comparable with those of Table 2A. Creeksea and Bush shore, about a mile, respectively, to the east and west of Burnham received the heaviest settlement indicating that this area, which is close to that on which Table 2A is based, represents the most heavily infested part of this river. It is interesting also to note from Knight-Jones's data that the proportionate increases in number settling, between 1947 and 1949, were greater in the upper parts of the river (e.g. Fambridge and Shop laying), where settlements were small, than in the more heavily infested part of the river nearer to its mouth. This suggests that the original colonies were

TABLE 2A. TOTAL ANNUAL SETTLEMENTS OF *ELMINIUS MODESTUS* IN ESSEX AREA AS TOTAL SPAT RECORDED PER SQUARE CENTIMETRE

Year	Settlement at low water	Settlement on raft	Location
1946	97	—	West Mersea
1947	28	1·9	Burnham-on-Crouch
1948	165	3·9	„
1949	531	59·4	„
1950	284	421	„

TABLE 2B. TOTAL ANNUAL SETTLEMENTS OF *ELMINIUS MODESTUS* BASED ON RECORDS GIVEN BY KNIGHT-JONES (1952)

Allowance has been made for the fact that his records do not cover the whole of the settling season. The figures below are therefore estimates only but probably correct in orders of magnitude. Units, number per square centimetre per season.

Locality	1947	1948	1949
Fambridge	0·2	42	160
Purleigh	—	55	53
Althone creek	—	4·7	—
Creeksea	—	27	410
Bush shore	72	270	470
Broadrakes	—	11	167
Shop laying	0·8	12	260
Paggleshain pool	—	3·1	—
Roach mud	—	0·75	9·8
Mean	24	47	219

probably located near the mouth, and that the species spread upstream, towards Fambridge and Battlesbridge, as the substrata available near the mouth became fully covered. Intertidal observations indicated that there was an increase in density of adults corresponding to the increase in numbers setting during the same period. For example in 1947 the barnacle population on wooden piles was composed of 10–15% *Elminius* and 85–90% *Balanus balanoides* at mean tide level. On small scoured stones from which barnacles were constantly removed by abrasion *Elminius* already constituted about 90% of the population. By 1949 *Elminius* everywhere outnumbered *Balanus balanoides* several times, and was practically the only barnacle found on small stones on the foreshore.

Similar increases were noted at Whitstable and Ramsgate between 1947 and 1951. On the other hand, no significant changes were seen elsewhere on the south coast, although observations were made at a number of places such as Hastings, Bexhill, Eastbourne, Brighton and Bognor during 1947–48 and between 1953–54. In sheltered areas, particularly those with wood and stone substrata, *Elminius* was everywhere abundant, but on more exposed parts of the coast, especially on wave-cut chalk reefs, such as those near Beachy Head and Seaford, *Elminius* was present only in scattered groups and was less common than *Balanus balanoides*.

Elminius has spread northward along the east coast during the past 10 years, as shown in Fig. 2. In 1947 it was fairly common as far north as Lowestoft, with isolated individuals recorded on the shores of the Wash at Brancaster, Hunstanton and Skegness. During 1948 and 1949 extensive settlements occurred in the Wash, so that by the end of 1949 *Elminius* had become the dominant intertidal species. The rivers entering the Wash were also heavily infested. In 1950 *Elminius* was abundant at such places as Kings Lynn, Holbeach and Fosdyke. It continued to spread northward. In 1948 isolated individuals were already present on piles at the extreme end of the pier at Cleethorpes, though none were to be found on the foreshore at Mablethorpe and Sutton-on-Sea. In 1950–51 *Elminius* became common along the whole of the Lincolnshire coast, as far as the entrance to the Humber at Cleethorpes. A single individual was found at Hornsea in 1953, but a further search in 1955 failed to reveal any *Elminius* on the Holderness coast, save within the Humber itself, a small number having been found at Paull and Kingston-upon-Hull. The only record of *Elminius* north of Hornsea is of one individual on a mussel shell from Blythe (Bull, 1950). This was evidently only a transient settlement, since it has not been followed by any general invasion of the area.

The advance of *Elminius* has therefore been halted abruptly at the mouth of the Humber. Three possible factors may be put forward to account for the failure of the species to spread further. (i) The residual currents flow southward off the Holderness coast, thus opposing the dispersal of larvae northward (Tait, 1938; Edgell, 1943). (ii) There are no rock substrata, and little or no artificial substrata suitable for barnacles for some 30 miles of coast north of Spurn Head. The abrasion on this part of the coast is moreover very severe. At Withernsea abrasion appears to have prevented anything but *Enteromorpha* sp. from attaching to the groynes even towards low-water mark. (iii) The Humber, unlike the Wash, is not a suitable area for *Elminius*, probably on account of

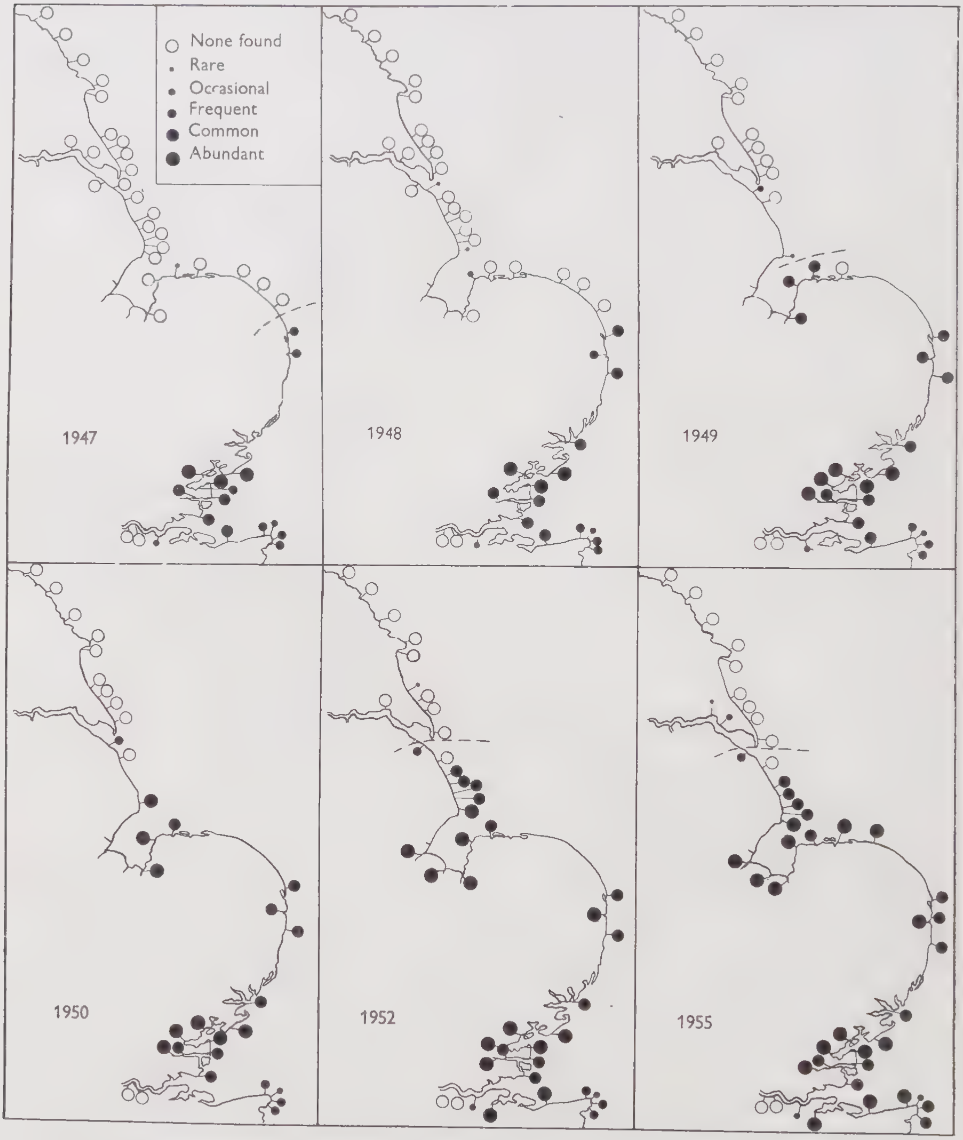
○ None found
• Rare
• Occasional
● Frequent
● Common
● Abundant
1947
1948
1949
1950
1952
1955

Fig. 2. Changes in the distribution of *Elminius* on the east coast from 1947 to 1955.

the pollution of the estuary. *Elminius* has not set up a dense population there, as it did in the Wash, and so may not be able to produce enough larvae to bridge the unfavourable Holderness coast.

It should be mentioned that many parts of the Yorkshire coast north of the Humber, particularly such places as Bridlington and Whitby, appear intrinsically suitable for *Elminius*, though sea-water temperatures north of the Humber are low, not usually exceeding 13–14° C in summer, and therefore unfavourable for rapid breeding.

SOUTH-WEST ENGLAND

In 1946 *Elminius* was common all along the south coast as far west as Poole Harbour. It was then rather rare at Swanage, and absent from the greater part of the Dorset coast, except for a possible centre discovered in 1947 at Weymouth (Fig. 3). By 1952 *Elminius* had become common at Swanage and Weymouth, and had appeared in smaller numbers in many of the intermediate anchorages, such as Kimmeridge Bay, Lulworth Cove, etc. Nevertheless, it has not spread westwards from Portland Bill. Apart from three specimens taken in West Bay in 1948, none has been found between Portland and Exmouth, despite repeated visits to the area. Whatever population once existed, none was found in West Bay in 1950 or in 1953.

Beyond the River Exe, *Elminius* is for the greater part confined to estuaries and drowned valleys, and absent from the open coast. Its first appearance in the area was at Plymouth in 1946. The following year an entirely separate colony was reported from the Helford River (Knight-Jones, 1948), and scattered individuals were found in Torbay late in 1947. It has since appeared in all the estuaries that have been examined between Helford and the Exe, but as it has not settled on the open coast, save as scattered individuals, it has spread somewhat erratically from one river estuary to another. For example, it became common to the west of Plymouth, in the rivers Fowey and the Looe in 1949, and to the east, in the Yealm (1948), Erme, Avon, Salcombe and Dart (1949). The Teign and Exe estuaries were colonized later, in 1949–50 and 1951, respectively. It therefore seems likely that Plymouth was the original centre of this dispersal, since the species appeared there as early as 1946 and achieved, by 1950, an average density of from 0·5–2·0 individuals per square centimetre on rocks and piles below mid-tide level in the rivers Plym and Tamar. Its colonization of these rivers may have been made easier by the widespread reduction in numbers of *Balanus balanoides* whose niche it has apparently filled (Southward & Crisp, 1952, 1954).

A significant feature of the spread into all the above rivers has been that the denser populations appeared first, and continued to increase, some distance upstream of the mouth. Gweek and Porth Navas in the Helford River and Greenway and Galmpton on the River Dart are good examples of sites where these early colonies developed, as shown in Fig. 4. The colonies in

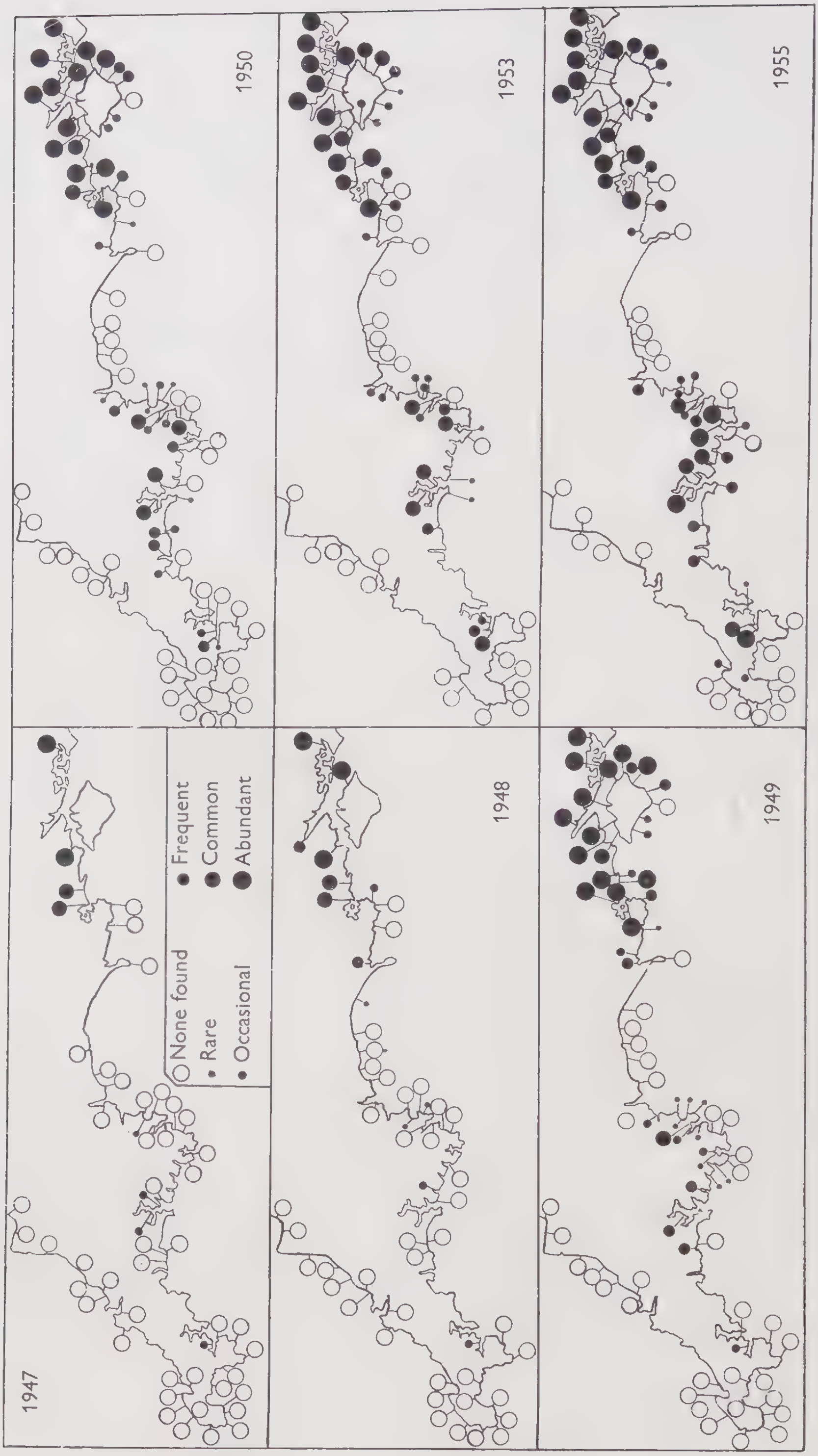
1947
1948
1949
1950
1953
1955
○ None found
• Rare
● Occasional
● Frequent
● Common
● Abundant

Fig. 3. Changes in the distribution of *Elminius* on the south-west coast from 1947 to 1955.

each estuary must therefore have become established and increased independently.

The spread of *Elminius* westwards from the Helford River into Penzance Harbour and on to St Ives occurred between 1951 and 1955, some years after it had first been found in the Helford River system.

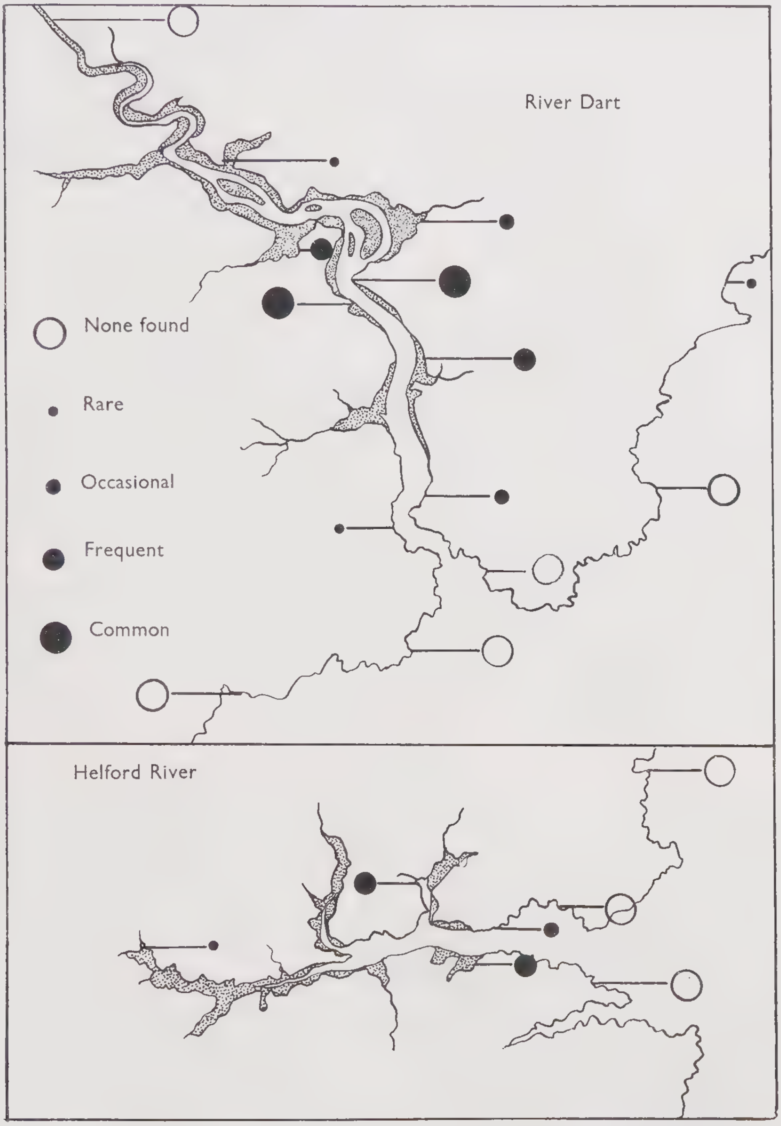
River Dart
None found
Rare
Occasional
Frequent
Common
Helford River

Fig. 4. The distribution of *Elminius* in the River Dart in 1949 (above) and in the Helford River in 1950 (below) to show where the first colonies develop in this type of estuary.

THE BRISTOL CHANNEL

The colonization of the Bristol Channel probably commenced at about the same time as that of Plymouth Sound. It arose as an independent population separated from those farther south by the unfavourably exposed coast of north Cornwall, which remains still for the greater part free of *Elminius* (Fig. 1). The earliest records were in two distinct areas. A strong but very local colony was found late in 1947 in Milford Haven, and isolated specimens were found in the same year on both the north and south banks of the upper reaches of the Channel (Fig. 5) (Bassindale, 1947; Purchon, 1947). In 1949 a careful search by Mr A. H. N. Molesworth and myself revealed a widespread occurrence of the species throughout the Channel. In the region of Penarth, Watchet and Weston-Super-Mare individuals occurred regularly, but at densities of only a few per square metre. They were rarely close enough to fertilize one another. Even more sparsely scattered populations were found west of Nash Point, in such places as Llanelly and Swansea, and at scattered points on the Somerset coast. In Milford Haven the species was becoming well established, with small groups of breeding individuals. A further survey in 1952–53 showed that great changes had taken place. *Elminius* was then common throughout Milford Haven, having almost reached the entrance by 1951, and along the whole of the South Wales and Somerset coasts, to Tenby and Lynmouth, respectively. The population in Milford Haven was therefore still separated from that in the rest of the Channel by the exposed piece of coast stretching from St Gavan's Head to Angle. A recent invasion of the Taw–Torridge estuary is similarly separated from the Bristol Channel by the exposed coasts from Lynmouth to Braunton, where the species remains very sparse.

So far as is known no spread of *Elminius* has occurred north of Milford Haven, so that the southern part of Cardigan Bay, including Fishguard, remains free of the species.

THE IRISH SEA

The colonization of the Irish Sea has afforded an opportunity for more critical investigation, since only a year or two elapsed between the introduction of *Elminius* and its limits being fully surveyed. *Elminius* was first observed in the Irish Sea in 1950, when individuals measuring up to 1 cm in diameter were found on the Lancashire coast. These must have settled at least as early as the middle of 1949. The whole of this coast south of Fleetwood had been searched by Dr P. N. J. Chipperfield in 1948, and he failed to record a single individual. This suggests that the settlements observed in 1950 dated from late 1948, and that the initial centre was in the Morecambe Bay area. Fig. 6 shows in detail how the population spread from Morecambe Bay. Its advance southward along the Fylde was rapid, and was continued westwards, along the North Wales coast, at a rate of some 30 km each year. Its advance northward along the Cumberland coast was less rapid until it reached the Solway, whence it appears to be spreading westwards more rapidly. No doubt the extensive sands of the Fylde were beneficial in producing summer temperatures higher

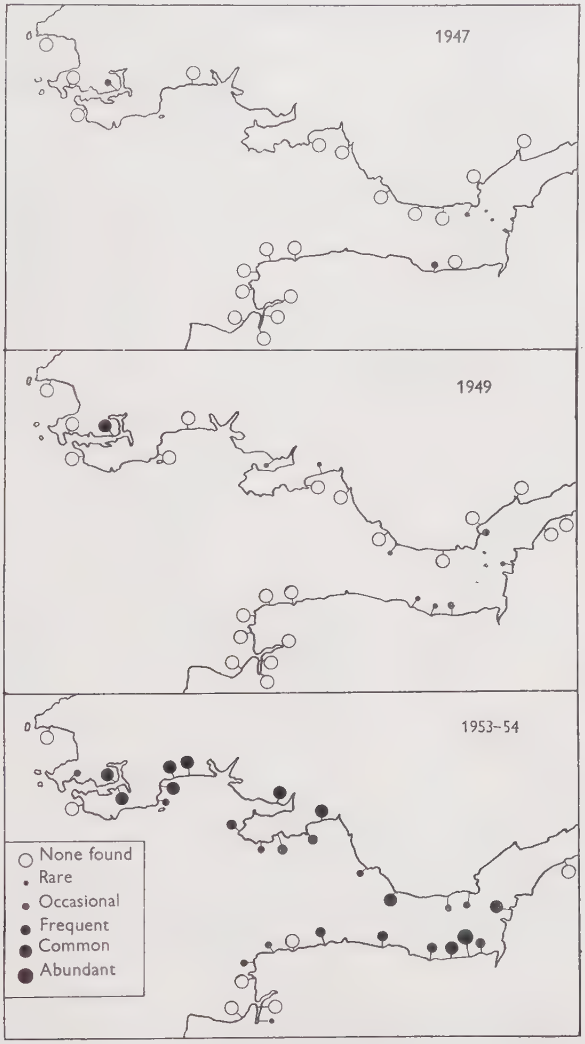

Fig. 5. Changes in the distribution of *Elminius* in the Bristol Channel from 1947 to 1954.

than those of the Cumberland coast, apart from the Solway. Perhaps more important, however, was the influence of residual drifts which are believed to flow southward along much of the Cumberland and Lancashire coasts and westwards along the Galloway and North Wales coast (Williamson, 1956).

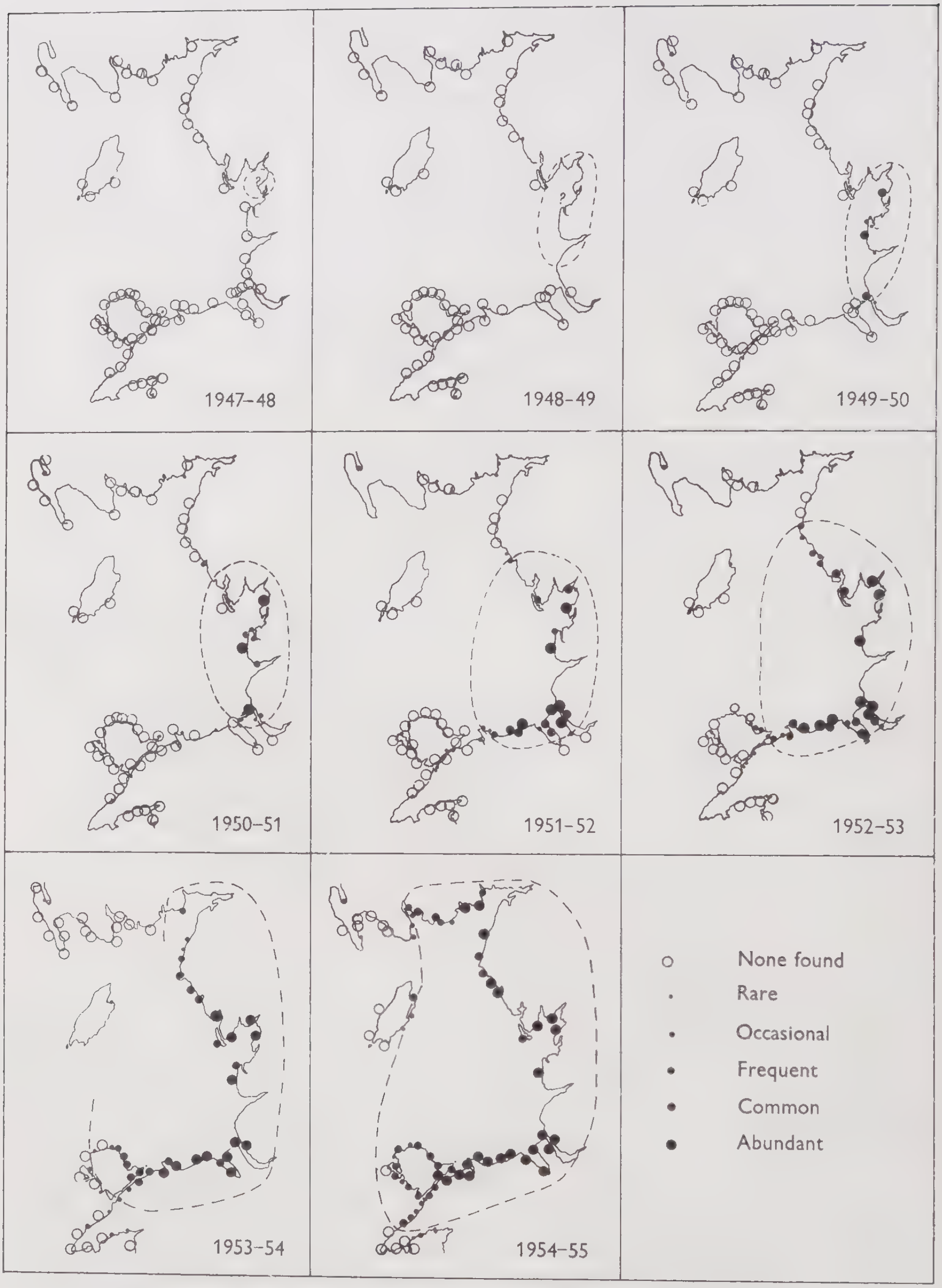
1947–48
1948–49
1949–50
1950–51
1951–52
1952–53
1953–54
1954–55
○ None found
• Rare
• Occasional
● Frequent
● Common
● Abundant

Fig. 6. Changes in the distribution of *Elminius* in the Irish Sea from 1948 to 1955.

Elminius has not advanced uniformly. Its advance has shown different patterns under differing hydrographical conditions. Along the north coast of Wales, from Rhyl to Bangor, the advancing population formed at any point of time a clearly marked front extending along a short stretch of the shore. Within a distance of about 20 km the density of the population fell from more than one individual per square centimetre to only a few per square metre. Fig. 7, based on counts made on areas colonized by *Elminius*, shows how this front spread along the shore year by year. The proportion of young individuals was greater at the advancing edge of the front, particularly during August and September, when the spatfall often covered the greater part of areas left bare by other species during the summer. Thus, it was quite usual to find that where *Elminius* was present only as scattered adult individuals in one year, it formed

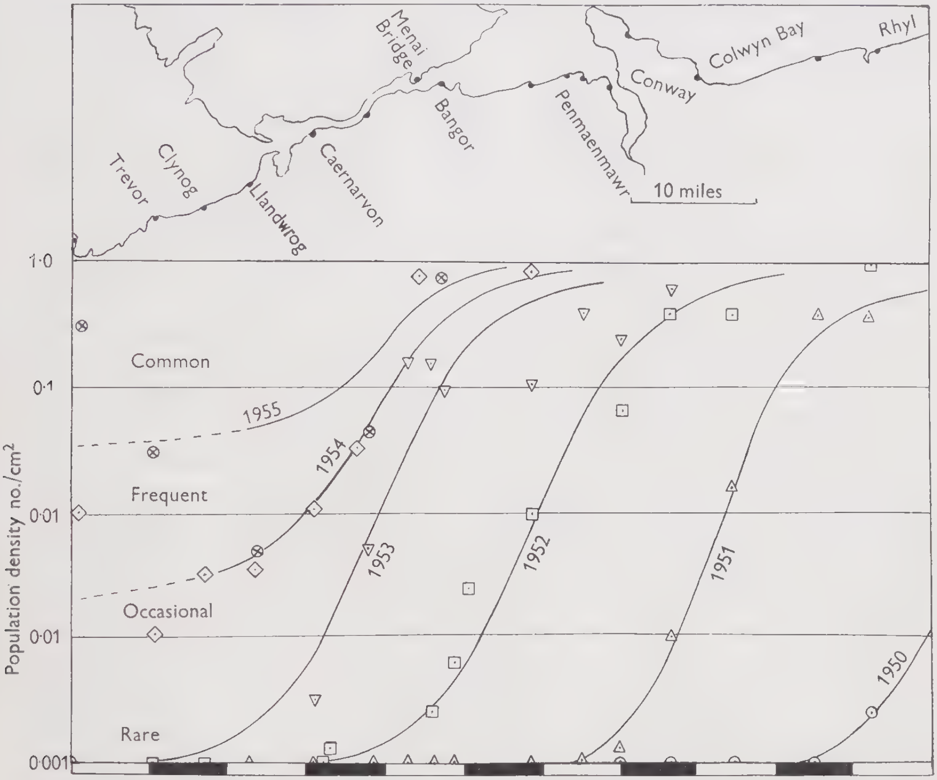

Fig. 7. The progress of *Elminius modestus* along the north coast of Wales. The upper part of the figure shows the coastal topography and the places at which regular counts were made. The lower part of the figure shows graphically the annual changes in the population density of *Elminius* (vertical axis) drawn to the same scale along the horizontal axis. Bottom scale represents intervals of 10 km.

a complete cover on all bare areas during the following season. For example, early in 1951 none could be found on the pier or elsewhere at Colwyn Bay, but by the end of the season the pier surfaces had acquired a density of $0{\cdot}04/\text{cm}^2$ of adult *Elminius* surrounded by spat which grew to maturity the following spring and reached a density of $0{\cdot}3/\text{cm}^2$ in July 1952.

The spread of *Elminius* round Anglesey to the Lleyn peninsula (Caernarvonshire) followed a different pattern. The well-marked front disappeared beyond Moelfre along the north coast of Anglesey and beyond Menai Bridge in the Menai Straits (Fig. 7). New centres appeared almost simultaneously and independently in bays and harbours along the north and west coasts of Anglesey and the Lleyn Peninsula. These centres consisted of small and very local patches of *Elminius* usually of density $0{\cdot}1\text{–}1{\cdot}0/\text{cm}^2$. The largest area infested was Holyhead Harbour, where a small number of *Elminius* had been recorded previously, but had shown no marked increase hitherto. The coast between these centres carried for the most part only isolated individuals, their density varying inversely with exposure to wave action.

The coastline to the west of Bangor differs from that to the east in three main respects. First, it is less shelving, and so the waves break directly on the rocks, the wave-crash being particularly severe on the north-west of Anglesey. Secondly, the tidal currents which flow through the Menai Straits and round the north coast of Anglesey are stronger than those which affect the coasts lying to the east of Bangor. Lastly, there are more extensive stretches of sand and gravel to the east of Bangor, and these shores, with the possible exception of the Orme, suffer more scour in consequence and have little algal cover. By contrast the north and west of Anglesey, the Menai Straits, and the Lleyn Peninsula have an exceptionally rich algal flora.

All three factors probably influence the spread of *Elminius*. The greater exposure to wave action and the denser algal cover restrict favourable habitats to silty bays and harbours. The greater tidal flow disperses the larvae more widely, and therefore more sparsely. Hence, localized populations spring up in suitable places over a wide area.

Since 1953 isolated individuals have been found from time to time on the north coast of Cardigan Bay. In 1955 this coast was carefully examined and the only incidence of the species in significant numbers was found at Pwllheli. It was present on stones in the harbour at a density of twenty to fifty individuals per square metre, some of them breeding. Some local fishing boats laid up on the beach had acquired a settlement of about the same density. It is not clear, therefore, whether these vessels introduced *Elminius*, or whether it has spread through Bardsey Sound by natural means.

By the end of 1955 the spread of *Elminius* westwards from the Solway along the south coast of Scotland had reached the Isle of Whithorn, and a single individual was found at Drummore near the Mull of Galloway. A small settlement which must almost certainly be regarded as a separate population has persisted at Stranraer since 1950 (Crisp & Molesworth, 1950). In 1953 and 1955 *Elminius* was confined to the same part of Stranraer harbour; its numbers had dwindled, and nearly all the specimens were old ones with corroded shells. Subsequently, it was discovered that a ship-breaking yard which previously existed on the east bank of Loch Ryan had been out of operation since 1949. Possibly ships awaiting breaking up had liberated larvae, giving an initial settlement in the Loch, but these were never in sufficient density to give rise to an expanding population. A more recent report of a single specimen of *Elminius* from the Clyde (Connell, 1955) has not been followed by further reports of its presence in that area. Occasional individuals are found not infrequently some 50 or 60 miles from the main stocks. Whether they are carried there by ships or by exceptionally favourable eddies is not certain.

An isolated record of two individuals was made by Dr Southward at Ramsey in the Isle of Man early in 1952, but no further specimens were found there in 1953. However, by 1955 a centre of dispersal had clearly been established at Ramsey and was spreading southward; moreover, some of the individuals were probably at least a year old.

Ramsey is the most northerly point in the Isle of Man where suitable conditions exist for *Elminius*. Its arrival there, approximately 1 year after it had colonized the Solway, and simultaneously with its appearance at Whitehorn and Drummore, suggests that it had been carried by natural means across a sea barrier of some 25–30 miles. Crisp & Southward (1953) believed that this distance was near the critical limit for the spread of this species by normal water movements adjacent to a shoreline. At that time it was separated by a distance slightly exceeding 30 miles, and the numbers of larvae arriving at Ramsey were apparently not sufficient to start a colony.

INVASION OF THE MAINLAND OF EUROPE

Apart from records off the Dutch coast (den Hartog, 1953) the information regarding the colonization of the coasts of Europe remains scanty, and is insufficient for definite conclusions to be drawn.

The first record for the mainland of Europe was from the Kijkduin district in south Holland (Meulen, 1946). Its presence was subsequently reported by Boschma (1948). It has generally been assumed that the species was carried by ship from Britain and made its first appearance in the region of the Hook of Holland. It spread steadily northwards, reaching the southern end of the Zuider Zee by 1950, and the northernmost Friesian islands by 1951 (den Hartog, 1953). It was present in Cuxhaven in 1953 (Kühl, 1954). This migration, well documented by den Hartog, covered a distance of only 20–30 km in the first 2 years, but thereafter advanced probably at a steadily increasing rate of some 50–70 km a year, to reach Cuxhaven by 1953. The very rapid spread through north Holland and Germany is probably attributable to the residual current flowing through the Straits of Dover and turning eastwards past the Friesian archipelago and the north German coast (Carruthers, 1930).

According to den Hartog (1953) *Elminius* spread even more rapidly from the Hook of Holland southwards across Belgium and France. In a subsequent publication (1956) den Hartog realized that the occurrence of *Elminius* in France was too widespread to be considered as having originated from Holland, and suggested that ships had carried the species to Normandy from England during the invasion of Europe. Bishop (1954) and Bishop & Crisp (1958) have described in detail the distribution of *Elminius* in France in 1953–54. They show that there were at least two distinct population centres, one in Brittany, and another, extending eastwards from the north coast of Cotentin, continuous with the Dutch population. In a more detailed consideration of the factors involved in dissemination, notably the influence of the residual current, Bishop & Crisp (1958) have arrived at the conclusion that the two French populations were established independently, and that the eastern population centre probably merged with the Dutch population on the Belgian coast approximately in 1949–50 (see Leloup & Lefèvre, 1952). A further centre has now developed in South Brittany between Concarneau and Lorient (Crisp, 1958), and another was discovered in 1955, at a great distance from all existing stocks, in north-west Spain (Fischer-Piette & Prenant, 1956).

MEANS OF DISSEMINATION

A species invading new territory may follow either of two possible courses. It may spread only at the boundary of the existing population, by natural processes of dispersal, or it may be carried a long distance by means of a vector and so establish a new centre of dissemination. It will be useful to refer to the former as marginal dispersal, and the latter as remote dispersal.

Elminius has clearly spread by both means. We shall therefore consider the limitations governing the two processes.

MARGINAL DISPERSAL

The process of marginal dispersal demands ideally a suitable coastline with closely spaced objects such as piers, breakwaters, and boulders available for settlement. Most of the north coast of Wales, between the Dee and Anglesey, fulfils these conditions. Fig. 7 shows the population density at various times, based on sample counts on various substrata. The vertical axis is given for convenience on a logarithmic scale. It can be seen that at any given time, the population density falls with increasing distance from the main stocks, sharply at first, then less steeply, and finally asymptotically to the horizontal axis. This pattern of distribution at the population boundary would be anticipated if dispersal were a random process, whether the population were a stable or an expanding one. Because the population extends at ever-decreasing density beyond the obvious boundary, occasional records may be made at considerable distances, perhaps 40 or 50 miles in some instances, from the main population centre. Indeed over these wide areas where the animal is scarce records will depend as much on the diligence of the observer as on the density of the species. For this reason the practice of defining the boundary of a species as the point most remote from the main population at which an individual has been found is unsatisfactory. An objectively and accurately defined boundary can only be set by means of distribution contours (isopleths) such as those shown in Fig. 7. Any isopleth may be chosen, but the position of the boundary is determined most accurately where the isopleths are closest together and where the abundance of the species is sufficient to allow counts to be made. In Fig. 7 the choice is clearly between a density of 0·1 and 0·001/cm^2.

When measuring the rate of dispersal year by year the same isopleth must obviously be used for comparison. Due to irregularities in the distribution and in the environment, its position will not always be as clearly defined as in Fig. 7; nevertheless an estimate of the position of the isopleth should be made.

Table 3 summarizes existing information on the rate of progress of *Elminius* by marginal dispersal. The British records are based on the movement of the point of density 0·1/cm^2 on substrata favourable to the species. The table records only the results from areas where the limits were made certain from time to time by searches up to and beyond those parts of the coast known to have been invaded.

The rate of advance along different coasts, when taken over a number of years, is usually of the order of 20–30 km per year. The exceptions are confined to coasts influenced by strong residual currents. For example, the eastward drift along the Dutch and German coast accelerated the spread considerably; the southerly drift along the north-east coast of England appears to have arrested it altogether. With a strong and favourable drift, the relatively small numbers of larvae produced at first by an initial colony may be spread out so thinly and to such great distances that they escape unnoticed. As the population increases in density and in extent the larval output may become sufficient to supply the current with enough larvae to prevent their being unduly dispersed. An increasing rate of advance would then accompany the expansion of the initial population, as occurred off the Dutch coast.

REMOTE DISPERSAL

During marginal dispersal a fresh area is colonized from a large well-stocked area nearby, and becomes in turn a source of larvae for further dispersal. The supply of larvae is therefore practically unlimited; the chief factor limiting the spread of the species is the distance which, on average, a larva will be carried during its planktonic life. In remote dispersal, however, the number of larvae introduced is limited. Even under the best conditions, as, for example, when an old hull covered in barnacles is laid up, the actual numbers of larvae set free must be very small compared with those which would be produced on a neighbouring well-stocked shoreline. The small number of larvae available in a given area is therefore a feature which in practice distinguishes remote from marginal dispersal.

TABLE 3. RATE OF PROGRESS OF *ELMINIUS* BY MARGINAL DISPERSAL

Region	Year	Estimated position of boundary	Distance covered (km)	Average rate of advance
North coast of Europe*	1946	Scheveningen	—	—
	1947	North of Oude Rijn	16	52 km/year (rate increasing sharply along Friesian archipelago)
	1948	North of Ijmuiden	33	
	1949	North of Petten	32	
	1950	Isle of Tessel	36	
	1951	Isle of Schiermonnikoog	92	
	1953	Cuxhaven	155	
East coast of England	1947	North of Yarmouth		
	1949	Hunstanton	75	33 km/year up to 1955. Advance ceased abruptly at the Humber
	1950	North of Skegness	26	
	1951	Cleethorpes	31	
	1951–55	No further advance	—	
West coast of England, southern front	1947	Morecambe Bay		
	1949	Mersey estuary	73	30 km/year
	1950	Point of Ayr	20	
	1951	Llandudno	36	
	1952	Red Wharf Bay, Aber.	25	
	1953	Holyhead, Port Dinorwic	30	
	1954	South of Porth Dinlleyn	25	
West coast of England, northern front	1947	Morecambe Bay		
	1949	Barrow-in-Furness	17	21 km/year (rate increasing as it spreads)
	1950	Millom	12	
	1951	Ravenglass	17	
	1952	Whitehaven	28	
	1953	Rough Firth	38	
	1954	River Cree	35	
South coast, eastward spread from Plymouth. (No clearly marked front, populations confined to estuaries and bays)	1947	Plymouth Sound		
	1950	Rivers Dart and Torbay	60–75	19·5 km/year
	1952	River Exe	35–20	

* From den Hartog (1953).

These few introduced larvae become dispersed by water movement, which will vary in magnitude with the topography, currents, tides and winds prevailing. Enclosed waters, especially long narrow estuaries carrying little freshwater drainage, will disperse the larvae less than open coasts with unrestricted lateral movement of the water by tides and winds. Thus, the introduction of the same number of *Elminius* into an area where the water exchange and dispersal is small will result in a more local and therefore denser spatfall. The tidal range is also of importance in controlling the amount of mixing of enclosed water with the open sea (Bishop & Crisp, 1958) and so the ultimate density attained by an introduced population.

This density of the initial population is of critical importance as the following experiments indicate. *E. modestus* spat were artificially isolated at various distances from their neighbours, and allowed to mature in an area almost free of the species (Brixham Harbour, 1949). Individuals within only 3 cm of each other were fertilized at the normal rate, but those spaced out at a distance greater than 4 cm never became fertilized (Table 4). The distance of 3–5 cm is equal to that of the fully extended penis. There is a tendency for the larger size groups with correspondingly longer penes to fertilize over a greater distance. The experiments indicate that cross-fertilization is obligatory in this species (cf. Crisp, 1954; Barnes & Crisp, 1956) and that individuals separated by a distance exceeding 5 cm will not produce any offspring. Observations in the field confirm this view (Table 4, columns 6, 7). Unless, therefore, the introduced cyprids, or those produced in the lifetime of the introduced individuals, can settle at such a density that a sufficient number of them are within 5 cm of each other, the colony will never establish itself. One may call this the 'critical breeding density'. If settlement were at random, and the conditions for maintaining the colony required that, say, 10% of the population reproduced, then, for this proportion to settle within 5 cm of each other, the critical breeding density would have to be somewhat in excess of ten per square metre. Owing to the gregarious tendency shown during settlement, a greater proportion will be in close proximity than would otherwise be expected, and this will improve the chance of successful colonization (Knight-Jones & Stephenson, 1950). Nevertheless, it is clear that the odds against an introduction must usually be very great. Success can only be achieved when large numbers are introduced into enclosed waters having little exchange with the open sea.

TABLE 4. EFFECT OF DISTANCE ON FERTILIZATION

The table gives, against the distance between individuals, the percentage which contained fertilized egg masses.

	Date and place of examination					
	Brixham transplants from Burnham-on-Crouch				Hunstanton	Neyland
Distance (cm)	Group A 1. v. 49 (%)	Group B 1. v. 49 (%)	Group C 23. v. 49 (%)	Group D 23. vii. 49 (%)	natural population 23. iii. 48 (%)	natural population 2. ix. 47 (%)
Controls, adjacent to each other	79	82	78	68	79	63
0 –1·0	70	80	83	—	69	55
1·0–2·0	72	22	67	—		50
2·0–3·0	50	0	75	27		20
3·0–3·5	30	0	30	73		0
3·5–4·0	0	0	25	20		
>4·0	—	—	0	0	0	0
Mean size (cm)	0·89	0·77	0·97	1·11	0·92	0·7

Dock areas, small harbours, and narrow estuaries with piston-like tidal movement are likely places for an introduction. Old ships brought from infected areas and laid up for some time or quantities of shellfish bearing spat would be the most likely sources of a successful introduction, since the *Elminius* thereby carried to the area will remain long enough to release successive broods of larvae (Crisp & Davies, 1955). Ships spending only a few days in the area are less likely to cause an introduction, for the actual numbers of larvae shed in so limited a time will be relatively small. Seaplane hulls and floats, though they occasionally become fouled by weed and are often treated with anti-fouling paint, rarely carry mature barnacles, and do not generally stay in one area for a long time.

There is no evidence that barnacles usually suffer harm when carried on ships, unless they have visited fresh or polluted water. On the contrary, the steady water movement provided by the slower classes of shipping is probably beneficial. Large gravid individuals can often be found on such vessels, indicating that growth has been rapid and breeding normal. Large ships from Australian waters also arrive from time to time fouled with *E. modestus*. Even when the species is absent outside the vessel, it is common to find it in the condenser boxes and ducts, in company with *Balanus amphitrite* and *Bugula neritina*, two other immigrant species to British waters.

BARRIERS RESTRICTING DISSEMINATION

We shall next consider the situation where parts of a coastline suitable for maintaining a population are separated by an unfavourable area. This barrier may be a rocky exposed section of the coast, or part of the open sea too deep for *Elminius*. Let us suppose one favourable area is already populated by the species but not the other. Fig. 8 illustrates this system qualitatively. The barrier is assumed to commence sharply at the termination of the existing population. The curve A shows the most probable form of the relation between the density of cypris larvae and the distance from the existing population from which they have been dispersed by water movements for the duration of their planktonic life. The situation is one which can be represented in its essentials by the equations of diffusion (cf. Skellam, 1955) in which the eddy diffusivity is responsible for random dispersal. It will be seen that at increasing distances from the parent stocks the density of larvae falls asymptotically to zero, as in the case of marginal dispersal (cf. p. 498 and Fig. 7). Since these larvae are ready to settle the potential settlement is represented also by the curve A. The maximum distance from the existing stocks, over which colonization is likely to occur, will clearly be determined by the distance at which the curve of potential settlement intersects the critical breeding density. This point is shown in the figure at A'. Fig. 8 also shows how the critical distance for colonization is influenced by the fecundity of the species K, the existing population density θ, and the degree of water movement represented by the eddy diffusivity ϕ (Sverdrup, Johnson & Fleming, 1942). An increase in K (or θ) will not affect the shape of the curve A, but will increase the larval density proportionately throughout as shown by curve B. The effect of increasing the degree of water movement (ϕ) is shown by curve C. The number of larvae available (area under the curve) is equal to that in A, but curve C is flatter and the critical distance increased in consequence as shown by the position of the point C'. It should be noted that the idea of a critical colonizing distance does not imply a clearly demarcated line beyond which colonization cannot occur under any circumstances. Owing to the assumptions involving probability in the above analysis, the effect of increasing the distance from the parent stocks is to diminish steadily the probability that a colony will be established. The critical colonizing distance is therefore definite only in relation to a given level of probability, and in the course of sufficient time increasing distances may eventually be bridged.

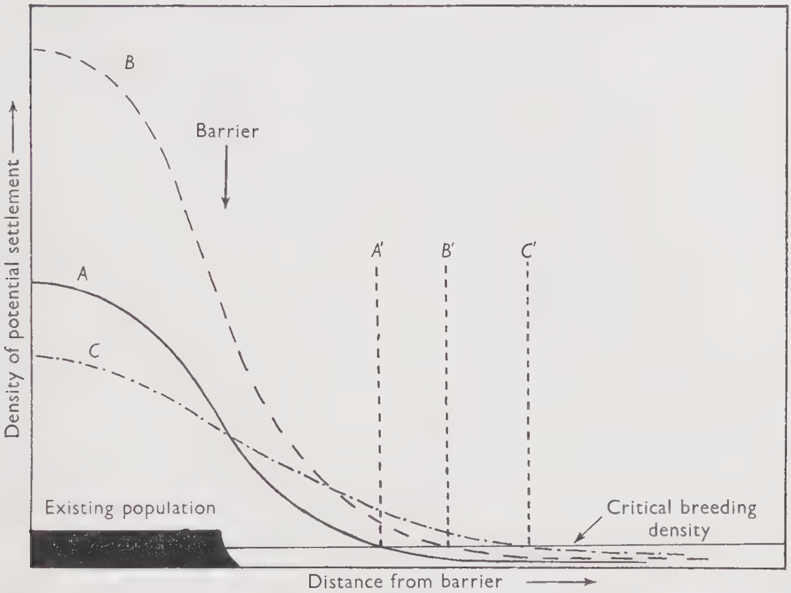

Fig. 8. Schematic representation of the influence of a faunistic barrier on dissemination of an animal with planktonic larvae. The existing population gives rise to larvae which, at the stage of potential settlement, are distributed according to diffusion theory as shown by curve A. Increasing the size or fecundity of the population will produce curve B, while increasing eddy diffusivity will modify curve A to curve C. The distance over which probable colonization may occur will be given by the intersection of curves A, B and C with the critical population level at which successful breeding can take place, that is at A', B' and C'.

It is rare to find a stretch of coast of any great distance wholly unsuitable or devoid of any substrata on which the species can settle. Probably the open sea is the most effective barrier, though even in the sea, fixed buoys may sometimes be present and provide regular oases. From the observations made in the northern Irish Sea area, it appears that the Isle of Man was colonized from a distance of some 20–25 miles away, but remained outside the critical range when the nearest *Elminius* were about 30 miles distant. This puts the critical distance for colonization in the order of 30 miles. Ireland, which is more than this distance from the coasts of England and Wales, had not been colonized by 1953, though a recent record shows that it has appeared in the south-west (Beard, 1957). The Channel Islands, separated by some 30 miles from infected parts of the French coast, are also reported to be free from *Elminius*. A more direct estimate of the critical colonizing distance may be made from an examination of Fig. 7. Since the settlement taking place in any season is derived in the main from the stocks which existed the preceding year, we may take the curve for, say, December 1951 as the density of parent stocks, and that for December 1952 as an estimate of the density of the spatfall derived from them during 1952. Putting the critical breeding density at two per square metre (this allows for gregariousness), and assuming the boundary of the population was at the isopleth $0\cdot1/\text{cm}^2$ in December 1951, the critical distance for colonization appears to be 29–30 miles, in good agreement with the above. There seem to be few estimates, relating to other species, with which this figure can be compared. Johnson (1939), however, found that the first-stage larvae of the intertidal crab *Emerita*, which were in the plankton for 3 weeks, were found only within 20–30 miles of the shore, while the last stage, with a planktonic life of 4 months, was carried some 150 miles out from the Californian coast. Since *Elminius* probably exists for 2 or 3 weeks in the plankton the eddy diffusivity appears to be of the same order of magnitude.

Probably the only extensive stretches of the British coast which are entirely devoid of substrata on which barnacles can settle are Chesil Bank, stretching from Portland to West Bay, and the Holderness coast of Yorkshire. There are, however, other places where *Elminius* has been temporarily or permanently halted; these are tabulated in Table 5. To what should the halt be attributed in these places?

Obviously the greater the proportion of unfavourable coastline, the smaller will be the numbers of larvae released and scattered along it, and the more likely will these larvae settle in unsuitable places or fail to reach the critical breeding density. Much of the Devon and Cornish coast is rugged and exposed, with few sheltered inlets. These conditions will diminish the rate of spreading of the species, for the majority of larvae produced will be wasted in unsuitable places. Another type of wastage may be important in such an environment, namely, the wastage of larvae offshore. Thorson (1950) draws attention to the calamitous wastage of larvae of littoral species from coasts where the prevailing winds and drifts are away from the land. Similar losses may occur at headlands which cause an offshore set of the current over a large part of the tidal cycle (Crisp & Knight-Jones, 1955), or where the tidal streams are particularly strong, as at Portland and Cap de la Hague. Larvae in such areas may have little chance of returning to the shore to settle. This wastage, taken together with the high proportion of unsuitable shoreline, would account for the halts in the spread of *Elminius* at Portland Bill, Cap de la Hague, the Lizard, and the Pembroke peninsula.

TABLE 5. BARRIERS TO THE PROGRESS OF *ELMINIUS*

Barrier	Possible causes	Distance separating suitable habitats	Period over which advance was retarded
Holderness coast	Lack of substrata. Opposing residual drift, low temperature	Hornsea–Humber	1953–
Portland Bill and Chesil Bank	Lack of substrata. Rocky headland. Strong tidal races. Opposing residual drift	Portland Harbour–West Bay Harbour	1947 or earlier–
Lizard and Land's End peninsula	Rocky and exposed headlands	Helford River–St Ives Bay (except for a few small harbours, e.g. Porthleven, Penzance)	1947 or earlier–1953 approx.
Pembroke Peninsula	Rocky and exposed headland. Strong tidal races	Milford Haven–Fishguard (except for small harbours such as Solva, Porthgain)	1947–
Irish Sea	Sea barrier	Cumberland coast–Isle of Man	1949–53
	Sea barrier	Holyhead–Dun Laoghaire	1953–
St George's Channel	Sea barrier	Milford Haven–Rosslare	1947–
English Channel	Sea barrier	Cap de la Hague–Channel Isles	1950?–
Straits of Dover	Sea barrier	Dover Harbour–Calais	1945 or earlier–1949?
Cap de la Hague	Rocky headland. Strong tidal races. Opposing residual drift	Cap de la Hague–Carteret	1950?–

The conditions on rocky coasts will in general be such that marginal dispersal can scarcely operate, or will do so only slowly. Larvae from sparse and scattered populations will be dispersed over a wide area, many being lost out to sea. Thus there will be a few larvae everywhere, but probably in most places not in sufficient density to breed. A few larvae liberated from craft lying up in a small creek or from the few individuals settled close enough together to breed, may add sufficiently to those being dispersed from outside to pass the critical breeding density. The area would then eventually become populated. Thus, where *Elminius* has spread from one harbour to the next, without appearing on the neighbouring exposed parts of the coast, as in south Devon, the Bristol Channel and west Caernarvonshire, it is possible that both marginal and remote means of dispersal have played a part. Where the distance traversed in one leap greatly exceeds 30 miles, however, it is reasonable to assume that the species has not spread naturally but has been carried by a vector.

PROBABLE HISTORY OF DISPERSAL

When first discovered in 1946 the species was already widespread in southern England. The two most likely areas for its original introduction from Australasian shipping would therefore be Southampton Water and the Thames estuary. Both are areas very favourable to *Elminius*, having high summer temperatures, and now support abundant populations. Of these two, Southampton is more likely to have been the original centre on the following grounds. First, Southampton Water is very enclosed, and because of the small tidal range it exchanges water only slowly with the sea outside. Secondly, there is little freshwater flow and less pollution there than in the Thames estuary. Thirdly, in the Thames all the docks are in areas too fresh or too polluted for survival of *Elminius* larvae, except possibly at Tilbury. In Southampton Water vessels are docked in sea water suitable for larval development. Lastly, though there are no continuous records for the Southampton area, we know that *Elminius* was still increasing in density in the Essex rivers from 1946–50.

No more than a guess can be made as to the time of arrival. The rate of spreading along comparable coasts of shingle and sand, such as north Holland, eastern England, and Liverpool Bay, lies between 52 km/year (with a favourable drift) and 30 km/year. There exists a general eastwards drift through the whole of the English Channel and Straits of Dover (Carruthers, 1930) and this probably extends to the shoreline as evidenced by the direction and movement of shingle bars by predominant wind and waves (Steers, 1946). A small contrary coastal eddy is shown by Edgell (1943) in Pevensey Bay. A rapid advance eastwards could therefore be assumed after the necessary time for establishment in Southampton Water. The many coastal defences in the area would also have assisted in providing substrata. Taking 50 km/year as a probable rate, then an introduction in 1939–40 would have reached the Thames estuary in 1945, and if the rate continued up the east coast it could have just reached Lowestoft by 1947. This timetable, however, appears only just adequate, and taking other factors into account an introduction by remote dispersal into the Thames estuary area probably in 1943 or 1944 seems more probable. In 1947 the species was more abundant in the area between the Thames and Harwich than on the Thanet coast, suggesting that the latter area was colonized after a population had been established in the Thames estuary. Furthermore, the Dutch coast was probably invaded as a result of remote dispersal between East Anglia and the Hook of Holland in 1946 (Bishop & Crisp, 1958). It is necessary, therefore, to assume that *Elminius* had been established in the Harwich area prior to 1946. Since the rate of advance northward from the Thames would not be accelerated by residual drifts, but on the contrary retarded since the strongest winds are from the north-east, it is not easy to account for the spread of *Elminius* to Harwich by 1945, unless it was already established in the Thames by 1943 or 1944. Making this assumption, the probable history may be summarized as follows.

Shipping conditions were exceptional at the outbreak of war in 1939. As convoys assembled, large numbers of vessels were anchored just offshore. This may have allowed the barnacles on Australasian shipping time to liberate sufficient numbers of larvae to colonize Southampton Water. The large concentration of shipping which then occurred might have given *Elminius* a unique opportunity. The following summer (1940) was a warm one, and would have assisted in building up stocks above the critical level for maintaining a population; from Southampton *Elminius* spread rapidly eastwards aided by the residual drift, and more slowly westwards. In about 1943 or 1944 a new centre was established in the Thames estuary, probably by remote dispersal, and the East Anglian and Thanet coasts were colonized from this centre.

The westward extension along the coast from Southampton was halted at Portland, and no further westward spread has taken root in Lyme Bay. Several distant centres, however, were set up to the west of Southampton, between 1944 and 1946, presumably by remote dispersal. These were in the Helford River (1946 or earlier), Plymouth Sound (1946), Milford Haven (1947), and possibly in another place further up the Bristol Channel. These two centres in South Wales may have arisen simultaneously, though independently. The centre in Milford Haven has remained isolated up to the present time by exposed headlands, while *Elminius* has steadily colonized the remainder of the coasts north and south of the Bristol Channel. Perhaps, however, the original breeding stocks were present in Milford Haven, and larvae were carried by the prevailing surface drift to coasts higher up the Channel.

A major centre was next set up in Morecambe Bay in 1948, from which the coasts of North Wales, Lancashire, Cumberland and Galloway have now been populated. Small centres, probably arising by remote dispersal, have been set up in St Ives Bay (1951–54) and Tremadoc Bay (1953–54).

On the continental coast there appear to have been four, possibly five, main centres. The invasion of the coast of Holland began near the Hook of Holland about 1946. For reasons given elsewhere, another centre was probably established in the estuary of the Seine soon after the invasion of Normandy in 1944 (Bishop & Crisp, 1958). A third centre in the Brest area has been described by Bishop from which other areas in south Brittany have been colonized in a manner similar to that of the invasion of the coasts of south Devon (Crisp, 1958). Judging from the rather slow establishment on rocky coasts of this type, the centre in the Rade de Brest was probably established at least 3 or 4 years earlier than the date (1953) when Bishop first surveyed the area. The river estuaries in the Morlaix–Roscoff area may represent other separate centres established at about the same time as that in the Rade de Brest. Much farther south yet another centre has been established in north-west Spain. Its discovery in 1955 (Fischer-Piette & Prenant, 1956) and its present rather limited area give it the appearance of recent origin, probably between 1950 and 1953. It may be distinct from recently reported settlements in Portugal (Fischer-Piette & Prenant, 1957).

The dates in which these centres have probably been established are shown in Fig. 9 .There can be seen to be some regularity in the order of their appearance, for the new centres have not sprung up at random. They seem to have arisen more readily in the vicinity of old ones than at great distances from them. Such is perhaps to be expected, since harbours are usually visited more frequently by craft from neighbouring localities than by craft from any other particular area. Local craft are also more likely to be allowed to accumulate fouling than are those which travel greater distances, and their shallower draught is more suitable for the settlement of an intertidal species. Larvae will thus be liberated continually into harbours once a neighbouring area is infected, to add to the numbers borne by water currents. It is therefore not easy to differentiate between remote and marginal dispersal in areas where suitable harbours are scattered and many local fishing vessels are operating.

ECOLOGICAL EFFECTS OF THE INTRODUCTION OF *ELMINIUS*

The introduction of a new species is bound to influence the balance existing between endemic species. A species occupying as dominant a place in its environment as *Elminius* may bring about profound changes. The main differences between *Elminius* and native barnacles are summarized in Table 6.

Elminius competes for space mainly with *Balanus balanoides*, which is the only indigenous intertidal barnacle on those parts of the British coast where *Elminius* thrives best, notably in south-eastern England. To a lesser extent it competes with *Balanus improvisus* and *B. crenatus* at low-water mark. *Elminius* ranges over a greater part of the intertidal zone than does *Balanus balanoides*, for small numbers grow at levels slightly above the highest *B. balanoides*, and it penetrates into the sublittoral, some 5 m below L.W.S. It is usually less common in the sublittoral, however, than *B. improvisus* and *B. crenatus*.

When *Elminius* first appears, the zone in which it settles is usually occupied by adults and spat of *Balanus balanoides*. Except in very muddy or brackish places, *B. balanoides*, which settles 2 months earlier than *Elminius*, initially covers the greater part of the available settling space, so that *Elminius* is often found attached to the upper parts of the shells of *Balanus balanoides* or even to the valves. As the spat of *Elminius* become more numerous they often form circlets of small grey barnacles round the apertures of large specimens of *Balanus*. Some *Elminius* settle regularly above the *Balanus balanoides* zone, almost as high as *Chthamalus* would be found if it were present.

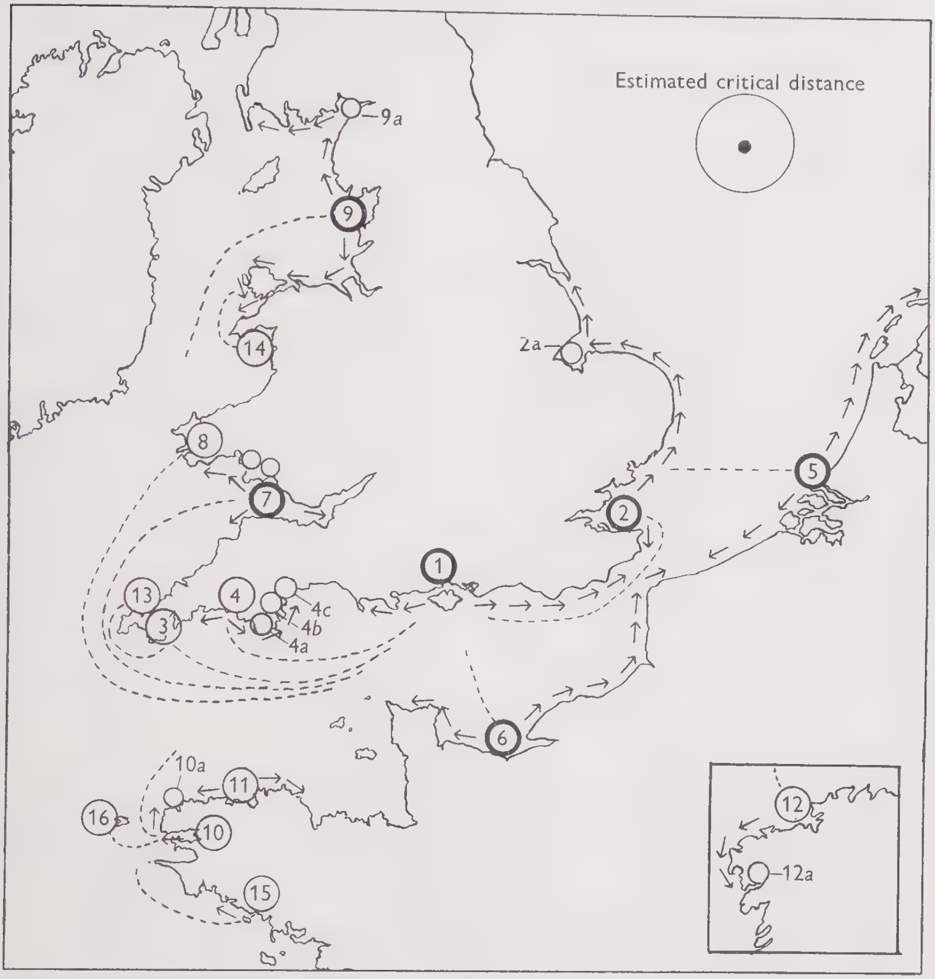

Fig. 9. Illustration of the probable order in which centres of dissemination of *Elminius* were established. Major centres responsible for the colonization of large areas of coast are shown by large dense circles. More localized centres also set up by remote dispersal are shown by large open circles. Subsidiary centres probably reached by marginal dispersal by small circles. Arrows show direction of marginal dispersal, dotted lines the probable routes of remote dispersal. Inset: north-west Spain

1, Southampton Water, ? 1940–43; 2, Thames estuary, ? 1943–44 [2*a*, the Wash, 1948]; 3, Helford River, ? 1944–46; 4, Plymouth Sound, 1946 [4*a*, River Dart, 1948; 4*b*, Salcombe, 1948; 4*c*, River Exe, 1951]; 5, South Holland, 1946; 6, Seine estuary, ? 1944–49; 7, Bristol Channel, 1946–47; [7*a*, Llanelly Bay, 1949; 7*b*, Swansea Bay, 1949]; 8, Milford Haven, 1947; 9, Morecambe Bay, 1948 [9*a*, Solway firth, 1953]; 10, Rade de Brest, ? 1944–52 [10*a*, l'Aber Wrach, l'Aber Beniot, ? year]; 11, Roscoff area, ? 1944–52; 12, north-west Spain, ? 1950–53 [12*a*, Noya, ? year]; 13, St Ives Bay, 1951–54; 14, Tremadoc Bay, 1953–54; 15, south Brittany, 1954–57; 16, Ile d'Ouessant 1956.

The extremely high intensity of settlement and the long breeding season allow *Elminius* to occupy any spaces left bare by *Balanus balanoides*. Sometimes in southern England over 100 spat only a few days old have been found per square centimetre of available surface. *Elminius* forms such a dense cover that spaces left by dead individuals, even in winter, are soon filled by the rapid growth of surrounding members of the species. It therefore becomes increasingly difficult for the cyprids of *Balanus balanoides* to find settling space, and they become displaced gradually by *Elminius* until only a small population remains. However, since *Balanus balanoides* is a larger species than *Elminius*, and continues to grow in height, these remaining individuals stand out further from the substratum and can fish a layer of water beyond the reach of the cirri of *Elminius*. They grow to a large size, measuring between 2 and 3 cm in diameter, and are particularly prominent on piers and jetties where the water is continually being renewed. Being no longer in such severe competition with their own species they are individually large, healthy and successful.

TABLE 6. ECOLOGICAL REQUIREMENTS OF *ELMINIUS*, COMPARED WITH THE NATIVE SPECIES

Species ...	*Elminius modestus*	*Balanus balanoides*	*Balanus improvisus*	*Balanus crenatus*	*Balanus perforatus*	*Chthamalus stellatus*
Season of settlement ...	May–Oct.	Mar.–Apr.	May–Sept.	Apr.–May	Aug.–Sept.	July–Sept.
Tidal levels occupied ...	M.H.W. to below L.W.S.	H.W.N. to L.W.S.	L.W.N. sub-littoral	L.W.N. sub-littoral	L.W.N. sub-littoral	H.W.S. to M.T.L. (sometimes to L.W.N.)
Tolerance of low salinity	+ + +	+	+ + +	+	+	–
Tolerance of silt	+ +	+	+ + +	+	+ +	+
Tolerance of low temperatures (below zero)	+ +	+ + +	+ + +	+ +	–	+
Tolerance of high temperatures (above 20° C)	+ + +	–	+ +	–	+ +	+ + + +
Tolerance of desiccation	+ + +	+ +	–	–	+	+ + + +
Resistance to mechanical damage	+	+ +	+ + +	+ + +	+ + + +	+ + + +
Mean rate of cirral beat at 20° C as beats per 10 sec (Southward, 1955*b*, 1957)	17–18	5–6	*ca.* 9	*ca.* 10	*ca.* 7	*ca.* 6

The process of replacement of the *Balanus* population by *Elminius* is relatively slow; for example, at Hunstanton, *Elminius* was introduced late in 1947, and present in small numbers chiefly on the parieties of *Balanus* on the pier piles in 1948. By 1949 it was abundant, and smothered all existing individuals of *Balanus*, though the latter were still present to a density of 2–3/cm^2. In 1952 some reduction in *B. balanoides* was noticeable, and those remaining were no longer close packed and columnar, but were in the main isolated, their density being about 0·7/cm^2. They were rather larger than hitherto. By 1955 the density of *B. balanoides* had fallen further to about 0·15/cm^2, most of these being between 2 and 3 cm across the base, and about 1·5–2 cm in height.

In the less saline parts of an estuary and below low-water mark, *Elminius* may replace *Balanus improvisus*. There was a reduction in the numbers of *B. improvisus* settling on experimental panels between 1946 and 1950 in the River Crouch, and, judging from plankton samples taken in 1938 in the Thames, a reduction has taken place in the numbers of *B. improvisus* larvae in the plankton. It will be especially interesting to observe this interaction if *Elminius* spreads to the Baltic, where *Balanus improvisus* is the dominant species. The competition between *Elminius* and *Balanus crenatus* is probably less important as there is less overlap in their habitats. They are found together only at low-water mark where the salinity is fairly high.

The effect of the introduction of *Elminius* is negligible on rocky exposed shores of the west and south-west, where the dominant intertidal barnacle is *Chthamalus stellatus*. Nevertheless, in sheltered inlets and estuaries of these coasts *Elminius* is of importance. In the Plymouth estuaries *Elminius* had in 1950 largely occupied the situations from which *Balanus balanoides* had disappeared during its decline (Southward & Crisp, 1954). Though *B. balanoides* has begun to return to the outer parts of this coast it has not re-established itself in the estuarine regions where *Elminius* remains dominant (Southward & Crisp, 1956). Its effect on the indigenous fauna within these estuaries has therefore been mainly at the expense of *Balanus balanoides* as on the coasts of south-east England. In many of the estuaries in the south-west where *B. balanoides* is not very common, *Chthamalus* and *Elminius* are found together, *Chthamalus* being able to penetrate up the estuary beyond the seaward limit of *Elminius*.

Elminius is microphagous and behaves like other barnacles (Southward, 1955*a*) utilizing a wide range of particle sizes and taking in both animal and plant material. It grows rapidly, sometimes reaching maturity (diam. 6–7 mm) in 8 weeks. The cirral beat at temperatures from 15–25° C is faster than that of indigenous species (Table 6). It continues to feed vigorously after maturity, and produces broods of nauplii at regular intervals (Crisp & Davies, 1955). Under optimum conditions broods may be liberated every 10 days, each brood containing about the same amount of living matter as is present in the soft parts of the parent. A dense population of *Elminius* therefore removes during summer a great bulk of suspended food and transforms it into larvae. These larvae are extremely abundant, often forming the dominant component of the plankton in May, June and July, in estuaries of south-east England. The nauplii probably feed mainly on particles less than $10\,\mu$ in size, as the diameter of the oesophagus is of this magnitude.

The replacement of a large proportion of the previously existing population of *Balanus balanoides* by *Elminius* may therefore have a considerable influence on other members of the marine fauna, for *Balanus balanoides*, unlike *B. improvisus*, is by no means the ecological equivalent of *Elminius*.

While both species as adults feed and so remove suspended food particles during summer, *Elminius*, judging from its rate of cirral beat (Southward, 1955*b*), is probably more efficient than *Balanus balanoides* at the highest temperatures. Hence, more food may be diverted to barnacle growth and so made unavailable to other microphagous forms. But by far the more important difference is that whereas *B. balanoides* accumulates reserves for winter breeding, *Elminius* rapidly converts the food into nauplii which enter the plankton during summer. Moreover, the large number of *Elminius* cyprids which develop and settle (see above) is a clear indication of the success of *Elminius* nauplii in obtaining the necessary food in the face of competition by other larvae having a similar diet. Consequently, there has been a great increase in the total number of summer nauplii, following the introduction of *Elminius* which must adversely affect the growth of the planktonic larvae of other animals which breed in summer, such as *Balanus improvisus*, *Polydora ciliata*, *Littorina littorea*, *Crepidula fornicata* and *Ostrea edulis*. *Ostrea* is harmed also in the early stages of growth as spat by competing for space with *Elminius* (Knight-Jones, 1948). Little is known of the relative ease with which different barnacles may be browsed by predators such as *Nucella lapillus* or *Asterias rubens*. The more delicate and fragile shell of *Elminius* might make it more readily devoured than indigenous species, and so allow these predators to be more successful.

Elminius tolerates the presence of silt and pollution probably better than any other species of British barnacle, with the possible exception of *Balanus improvisus* and in warmer situations *B. amphitrite*. In dirty harbours and muddy rivers within the intertidal zone it may have few or no competitors. On the other hand, on clean and especially on wave-beaten shores, *Elminius* has not displaced either *Balanus balanoides* or *Chthamalus stellatus*, and is indeed often very sparse on these habitats. Mechanical explanations at first suggest themselves, for the species is more fragile and might suffer more from wave crash or pebble pounding. Perhaps, it might be supposed, limpets, which are rare or absent from the usual haunts of *Elminius*, destroy any settlement on exposed shores. But these explanations are not entirely satisfactory. Exposed rocks very close to stocks of *Elminius*—Point Lynas, Anglesey, for example—may have numbers of *Elminius* settled on them which from their eroded appearance seem to have survived for some time. There is no marked preponderance of young individuals and spat in these places. *Chthamalus*, the larvae of which are as small as those of *Elminius*, settles successfully in spite of limpet browsing. It seems more probable, therefore, that the species is uncommon on exposed coasts because its larvae do not settle there, rather than because of high mortality. There are no definite facts to indicate whether *Elminius* larvae could develop in the clearer waters that surround more exposed coasts, but it seems probable that the more turbid water of tidal estuaries contains particles of the appropriate kind to nourish them, while offshore water in general does not.

When considering the influence of other organisms competing with

Elminius for rock space, algae probably rank equally in importance with animals (cf. Barnes & Powell, 1953; Southward, 1953). Where the water is sufficiently clear and where, as in shelter, limpets are few, the growth of algae prevents barnacles from colonizing much of the illuminated surface of rocks. Hence, barnacles may appear to avoid insolation (cf. Moore, 1944) and to collect in dark crevices under piers, or beneath boulders and overhangs. Thus, there exist in temperate waters two extreme situations in which rock surfaces are available for barnacle settlement; one where the rocks are so covered in silt, scoured, or affected by low salinities that algae do not greatly flourish, and the other where algae are kept down by the grazing activities of limpets and by heavy surf. *E. modestus* and *Balanus improvisus* are adapted to the former habitat, *Chthamalus* to the latter. In adapting itself to these turbid estuarine conditions it is possible that it has become nutritionally dependent on the type of food particles found in silty estuaries and is ill adapted to survive in clear water.

SUMMARY

Material collected prior to 1940 indicates that *Elminius modestus* was not present on British coasts at that time.

Elminius increased in abundance in south-east England from 1946 to 1950 and extended its range as far as the Humber, where it halted.

Its advance westwards along the south coast was similarly halted at Portland, but by 1948 independent colonies had been established in several of the river systems of Devon and Cornwall, in Milford Haven, and in the Bristol Channel.

The first populations in the Irish Sea were in Morecambe Bay. From there *Elminius* spread rapidly south and west along the north coast of Wales, and more slowly north and west towards Galloway, eventually bridging the sea to the Isle of Man.

Detailed observations showed that *Elminius* advanced along the uniformly favourable north coast of Wales as a definite front moving at a rate of approximately 20–30 km per year. Around Anglesey where tidal currents were stronger it appeared simultaneously in many scattered centres.

A distinction is drawn between marginal dispersal taking place under the influence of normal agencies at the boundary of an existing population, and remote dispersal due to an artificial or freak transport over a long distance. In the case of *Elminius* the maximum distance that is likely to be bridged by marginal dispersal in the absence of strong residual drifts is about 30 miles.

Elminius probably first appeared near Southampton, and was introduced into the Thames estuary area probably by remote dispersal. Thence it spread along the east coast and was transported to Holland. Its extension into south Devon, the Bristol Channel, the Irish Sea, and to the French coast must also be attributed to remote dispersal.

The main ecological effects of *Elminius* result from competition for space with *Balanus balanoides*. Since *Elminius* breeds in summer, its dominance has a profound effect on the composition of the summer plankton, greatly increasing the number of barnacle nauplii, presumably at the expense of other larvae.

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APPENDIX

TABLE 7

Key: A Abundant. All available surfaces well covered to 30 % of area or more. Adults at 1·0/cm^2 or more. C Common. Covering less than 30 % of available area, the majority within 1–2 cm of each other. Density 0·1–1·0/cm^2. F Frequent. Readily found, but about half the population within 3 cm of each other and so just able to breed. Spat not usually in evidence. Adults 0·01–0·1/cm^2. O Occasional. Very local and must be searched for, very rarely close enough to breed. Density 1–100/m^2. R Rare. Only a few isolated individuals found in an hours' search. Density below 1/m^2. N. None found after 1 hr search in suitable area.

Records of Elminius modestus

Place	Date	Density	Notes*
Dunbar	vi. 55	N	—
Berwick-on-Tweed	vi. 55	N	—
Blyth	x. 47	N	P.N.J.C.
	1949	R	Specimen on mussel, but no subsequent records here. H.O.B.
Whitley Bay	ix. 49	N	—
Cullercoats	ix. 53	N	—
Runswick	vi. 55	N	—
Robin Hood's Bay	ix. 50	N	P.N.J.C.
	1954	N	E.A.S.
Scarborough	vi. 55	N	—
Flamborough Head	vi. 55	N	—
Bridlington	vi. 55	N	—
Hornsea	vi. 52	R	1 specimen only
	vi. 55	N	—
Aldburgh	vi. 52	N	—
	vi. 55	N	—
Withernsea	vi. 55	N	—
Kilnsea	vi. 55	N	—
Paull	vi. 55	O	—
Hull	vii. 48	N	—
	vi. 52	N	Area unsuitable from pollution?
	vi. 55	R	—
Grimsby	vii. 48	N	Few suitable substrata
Cleethorpes	vii. 48	R	Single specimen at far end of pier
	ix. 50	O–F	Just self-maintaining population
	vi. 52	F	Less increase than expected
Saltfleet	vi. 52	(N)	No suitable substrata
Mablethorpe and Sutton-on-Sea	vii. 48	N	—
	vi. 52	C	—
Ingoldmells and Chapel Point	vii. 48	N	—
	vi. 52	C	—
Skegness	vii. 48	O–R	5 specimens on pier
	ix. 50	C–A	—
	vi. 52	A	Much commoner than *B. balanoides*
Fosdyke	vi. 52	A	—
Sutton Bridge	vi. 52	A	—
	vii. 55	A	—
King's Lynn	vii. 48	N	P.N.J.C.
	xii. 49	C	On mussels. G.D.W.
	vii. 50	A	Adults 3·5/cm^2. Spat fall up to 60/cm^2
	vi. 52	A	—
Hunstanton	viii. 47	N	—
	iv. 48	R–O	On pier piles only
	vii. 50	A	Heavy spatfall of 40–50/cm^2. Adults much commoner than *B. balanoides* and growing on them
	i. 53	C–A	A few damaged on pier after heavy storm, which caused widespread damage on east coast; much scouring of rocks and low substrata
	vii. 55	A	Only a few *B. balanoides* of large size (3–4 cm) on pier, 0·07/cm^2. Rest of area completely covered by *Elminius*
Brancaster	viii. 47	R	One small specimen only
	iii. 48	N	—
	vi. 49	F	Many small specimens on old wreck, off Scolt Head. A.H.N.M.
	ix. 49	C	Many small spat (up to 10/cm^2) on stones near main creek. P.N.J.C.
	vii. 50	C–A	Settlement local, two or three times as abundant as *B. balanoides.* Spat 30/cm^2
Wells	ix. 49	N	P.N.J.C.
	vii. 55	A	On mussels in creek
Sheringham	i. 48	N	Little suitable substrata. P.N.J.C.
	vii. 55	A	Confined to low water owing to scour
Cromer	i. 48	N	P.N.J.C.
Sea Palling	i. 48	N	P.N.J.C.
Great Yarmouth	i. 48	C	P.N.J.C.
Lowestoft	x. 47	F	On sheltered side of north jetty
	i. 48	F	P.N.J.C.
	v. 49	C	—
	xi. 51	A	—
	vii. 55	A	More restricted on open coast owing to scour. *Elminius* 98 % of population

* Initials refer to observers.

Place	Date	Density	Notes
Southwold	ii. 48	C	P.N.J.C.
	i. 53	C	—
Harwich	ii. 48	C	P.N.J.C.
Clacton	ix. 47	A	—
West Mersea	v. 46	C–A	Regular settlement throughout 1946
Maldon	ix. 49	A	—
Mayland	viii. 47	F	—
Steeplestone and Bradwell	viii. 47	A	—
Burnham-on-Crouch	i. 47	C	—
North Fambridge	viii. 46	C	On hull laid up above H.W. mark since July 1945
Hullbridge and Battlesbridge	ix. 49	C–A	—
Shoeburyness	ix. 47	C	—
Woolwich	ix. 47	N	Too much pollution
Erith	ix. 47	N	Too much pollution
Gravesend	x. 47	O	Limit of penetration of Thames estuary
Rochester	iv. 51	A ?	Had been abundant, but nearly all dead
Whitstable	ix. 47	C	—
	iv. 51	A	—
Birchington and Margate	ix. 47	F	—
	iv. 51	C–A	Abundant on piers (5/cm²) fairly common on chalk (0·5–1·0/cm²)
North Foreland	ix. 47	O	—
	iv. 51	O	—
Broadstairs and Ramsgate	ix. 47	F	—
	iv. 51	C	—
Dover	ix. 54	C	—
Folkestone	viii. 56	F	Less common than *B. balanoides*
Hythe	viii. 56	O	On groynes, few barnacles present, much abrasion
Rye Harbour	ix. 48	A	—
Fairlight	viii. 56	F	—
Hastings	viii. 48	C–A	—
	vii. 53	C–A	—
Bexhill	ix. 48	A	—
	vii. 53	C	—
Eastbourne	viii. 48	C	—
	vii. 53	A	—
Seaford	vii. 53	O	Much abrasion by shingle
Brighton	ix. 47	F	—
	iii. 49	F	—
Portslade and Shoreham	ix. 47	A	—
	iii. 49	A	—
Worthing	xi. 48	A	—
Littlehampton	xi. 48	A	Abundant on training wall of River Arun
	vi. 53	A	—
Bognor	xi. 48	C	—
	vi. 53	A	—
Chichester Harbour	vii. 44	F ?	Several spat on panel exposed for 3 weeks. H.G.S.
	vii. 45	A	About 30 spat/cm² of test surface. M.W.H.B.
	vii. 48	A	—
Portsmouth and Gosport	v. 44	?	2 specimens from boom defence vessel settled in 1943. H.G.S.
	1944	C ?	Numerous specimens on ship's hull. H.G.S.
	iii. 49	A	A.H.N.M.
Southampton	vii. 49	A	Intense settlement covering all substrata
River Test, Redbridge	xi. 48	F	—
Cowes	vii. 49	A	—
Ryde	viii. 48	A	—
	vi. 53	A	—
Bembridge	vii. 49	A	—
Culver	vii. 49	F	Less common on chalk than elsewhere
Sandown	vii. 49	A	—
	vi. 53	C	Less common than *B. balanoides*
Shanklin	vii. 49	F	—
	vi. 53	C	—
Ventnor	vii. 49	F	—
	vi. 53	F	—
St Catherine's Point	vii. 49	N	—
	vi. 53	R	—
Brook and Freshwater	vii. 49	O	—
	vi. 53	O	—
Alum Bay	vi. 53	O	—
Totland and Colwell Bay	vii. 49	C	Up to 2·5/cm² in sheltered areas
	vi. 53	C	—
Yarmouth, Isle of Wight	vii. 49	A	—
Fishbourne	vi. 53	C–A	—
Lymington	vii. 49	A	Present on piles withdrawn from the sea in July 1947
Milford to Highcliffe	vii. 49	(N)	No suitable substrata
Mudeford	vi. 49	A	—
Hengistbury Head	vii. 49	O	—
Bournemouth	viii. 47	C	—
	vi. 49	A	—
Sandbanks, Poole Harbour	vi. 47	C	Settlement of spat at rate of 3–5/cm²
	iv. 48	C	10–15 % of population
	vi. 49	C	—
Studland	vi. 49	A	—
	vi. 53	A	—
Swanage Bay	iv. 48	O	Absent beyond Peveril Point
	vi. 49	F	Absent beyond Peveril Point
	vi. 53	F	Rare beyond Peveril Point
Kimmeridge Bay	xi. 48	N	—
	vi. 53	F	—
Lulworth Cove	v. 49	R	—
	vi. 53	N	—
Osmington Mills	vii. 49	O–F	—
	vi. 53	O	—
Weymouth	iv. 48	F	5 % of *B. balanoides* population
	vi. 49	F	No perceptible increase
Portland Bill	vi. 49	N	—
	vi. 53	N	—
West Bay	xi. 48	R	5 small specimens in inner harbour
	vii. 49	N	—
	vii. 54	N	—
Seatown	vii. 49	N	—
Lyme Regis	xi. 48	N	—
	vii. 50	N	—
	vii. 54	N	—
Seaton	vi. 47	N	—
	xi. 48	N	—
	vii. 54	N	—
Sidmouth	vii. 54	N	—
Budleigh Salterton	viii. 47	N	—
	vii. 54	N	—
Starcross	ix. 47	N	—
	iii. 49	N	A.H.N.M.
	xi. 51	R	—
	iv. 54	F	—
Dawlish	v. 53	O	—
Teignmouth	viii. 49	O	Confined to River Teign estuary
	vii. 50	F	One specimen from Teignmouth pier
Torquay	ii. 48	N	P.N.J.C.
	vi. 49	R	—
	vi. 51	O–R	—
Paignton and Broadsands	xii. 47	R	—
	vi. 48	R	—
	vii. 50	R–O	—
	iv. 54	O	—

APPENDIX (*cont.*)

Place	Date	Density	Notes
Brixham Harbour	vii. 48	N	—
	viii. 49	R	—
	vii. 50	R–O	A self-maintaining colony at foot of breakwater
	xii. 51	O	—
	iv. 54	O	—
St Mary's Bay and Mansands	iv. 48	R	P.N.J.C.
	viii. 50	R	—
Dartmouth	iii. 48	N	—
	x. 49	R	P.N.J.C.
	iii. 50	O	G.W.R. Ferry
	iv. 54	C–A	Higher Ferry
Greenway, River Dart	ix. 49	C	P.N.J.C.
Galmpton	v. 48	N	P.N.J.C.
	iii. 49	O	M.W.H.D.
Stoke Gabriel	v. 48	N	P.N.J.C.
	iii. 50	O	—
Blackpool, Devon	iv. 50	N	—
Torcross	iv. 50	N	—
Charleton Bridge	iii. 50	C	—
	iv. 54	A	—
Kingsbridge	viii. 49	O	—
Head of Frogmore Creek, Salcombe	iv. 56	A	*Elminius* only barnacle. A.J.S.
Salcombe Harbour	iv. 49	R	—
	iii. 50	F	—
Salcombe, south beach	x. 48	N	—
	iii. 50	N	—
	iv. 54	O	—
Boit Head	vi. 49	N	P.N.J.C.
	iv. 57	N	—
Thurlestone	vi. 49	N	P.N.J.C.
	iv. 57	N	—
Bantham, River Avon	iv. 49	N	P.N.J.C.
	vi. 49	R	—
Ermemouth	viii. 49	R	—
	v. 55	F–C	A.J.S.
Steer Point, River Yealm	vi. 49	N	P.N.J.C.
Newton Ferrers	x. 48	R–O	—
	viii. 49	F	—
	iv. 57	C	—
Breakwater, Plymouth	ix. 51	F–C	A.J.S.
	xii. 54	F–C	A.J.S.
	ii. 56	C	Abundance confined to sheltered side of breakwater. A.J.S.
Rum Bay, Plymouth	viii. 48	N	P.N.J.C.
Tinside, Plymouth	v. 46	O	On mussels on old pier. P.N.J.C.
	xii. 46	O	G.W.R. dock
	xii. 54	C–A	A.J.S.
River Plym, Laira Bridge	iii. 50	C	—
River Tamar, near Saltash	viii. 49	F	—
	xii. 54	A	A.J.S.
Torpoint	viii. 49	C	—
Hole's Hole, Tamar	ii. 56	C	A.J.S.
Rame Head	i. 50	R	P.N.J.C.
	v. 52	R	J.H.O.
	x. 56	F–C	A.J.S.
Looe Beach	ix. 49	N	A.H.N.M.
	i. 50	O	None to east of Looe on exposed rock. P.N.J.C.
Looe River	viii. 48	N	P.N.J.C.
	ix. 49	F	A.H.N.M.
	i. 50	F	P.N.J.C.
Polperro	viii. 48	N	P.N.J.C.
Fowey	i 50.	F	—
Par	i. 50	O	—
	v. 55	F	—
Charlestown	i. 50	N	—
Falmouth	i. 50	N	—
	iv. 56	R	A.J.S.
Malpas	vi. 49	C	E.W.K.J.
Meanporth and Durgan	v. 50	N	—
Helford Passage	1947	O	Well established but not common. H.A.C.
	v. 50	O	—
Porth Navas	v. 50	F	—
	v. 55	C	A.J.S.
Gweek	v. 50	R	—
	v. 55	A	A.J.S.
Coverack	vi. 51	N	—
Lizard	vi. 51	N	—
	v. 55	N	A.J.S.
Porthleven	v. 50	N	—
	v. 55	N	A.J.S.
Prah Sands	v. 50	N	—
Penzance	v. 50	N	—
	v. 55	O	A.J.S.
Scilly	v. 55	N	A.J.S.
Mousehole, Lamorna, Treen, Sennen, Morvah, Zennor	v. 55	N	A.J.S.
St Ives	v. 50	N	—
	v. 55	O	A.J.S.
Hayle	v. 50	N	—
Porthcothan	viii. 48	N	D.P.W.
Padstowe	viii. 48	N	D.P.W.
Port Isaac	viii. 48	N	D.P.W.
	v. 53	N	—
Porth Gaverne	v. 53	N	—
	v. 55	N	A.J.S.
Tintagel	v. 53	N	—
Boscastle	v. 53	N	—
	v. 55	N	A.J.S.
Bude	v. 53	N	—
	v. 55	N	A.J.S.
Clovelly	viii. 49	N	P.N.J.C.
	v. 51	N	—
Westward Ho!	vii. 49	N	E.N.
	v. 51	N	—
	viii. 54	N	—
Barnstaple	vii. 49	N	E.N.
Appledore and Bideford	viii. 48	N	—
	vii. 49	N	E.N.
	vii. 52	R	E.N.
Saunton sands	vii. 49	N	E.N.
	viii. 54	N	—
Woolacombe	vii. 49	N	E.N.
	viii. 54	O	—
Ilfracombe	vii. 49	N	E.N.
	viii. 54	O	—
Combe Martin	v. 54	N	A.J.S.
Lynmouth	viii. 54	F	—
Porlock	viii. 54	F–C	—
Minehead	ix. 49	R	—
Blue Anchor	xii. 47	O	C.M.H.
	i. 48	R	P.N.J.C.
	ix. 49	R	—
	viii. 54	F	—
Watchet	xii. 47	N	C.M.H.
	ix. 49	O	—
	viii. 54	C	—
Kilve	viii. 54	A	—
Lylstock	v. 54	F	A.J.S.
Weston-super-mare	iii. 50	R	—
	v. 54	C	A.J.S.
Clevedon	iii. 50	N	—
Portishead	iii. 50	N	—
	v. 54	N	A.J.S.
Newport (Mon)	viii. 49	N	A.H.N.M.
Cardiff	viii. 49	N	A.H.N.M.
Penarth	viii. 49	O	A.H.N.M.
Sully Point	viii. 49	N	A.H.N.M.
Barry	x. 47	R	G.D.W.
	vii. 54	O	—
Rhoose and Aberthaw	viii. 49	N	A.H.N.M.
	vii. 54	O–R	—
Stout Point	viii. 49	N	A.H.N.M.

Place	Date	Density	Notes
Nash Point	viii. 49	R	A.H.N.M.
	vii. 54	C	—
Dunraven Castle	viii. 49	N	A.H.N.M.
Porthcawl	vii. 54	O	—
Port Talbot	viii. 49	N	A.H.N.M.
Swansea	viii. 49	R	A.H.N.M.
	vii. 54	C	—
Mumbles	viii. 49	N	A.H.N.M.
	vii. 54	F	—
Oxwich	viii. 53	F	—
Port Eynon	vii. 54	O	—
Worms Head, Gower	vii. 54	F	Mostly towards L.W.M.
Llanelly	viii. 49	R	A.H.N.M.
	viii. 53	C	—
Pendine	viii. 49	N	A.H.N.M.
	viii. 53	C	—
Amroth	viii. 53	C	—
Saundersfoot	viii. 53	C	—
Tenby	viii. 49	N	A.H.N.M.
	viii. 53	O	—
Freshwater, west	viii. 53	N	—
Dale Fort	xi. 51	O	Just appeared at H.W.N. tide level. J.H.B.
Neyland	x. 46	N	—
	ix. 47	R–O	—
	iii. 49	C	A.H.N.M.
	viii. 53	C–A	—
St David's	viii. 53	N	—
Porthgain	viii. 53	N	—
Goodwick and Fishguard	iii. 49	N	A.H.N.M.
Newport	iii. 49	N	A.H.N.M.
Gwbert	viii. 53	N	—
Aberporth	viii. 53	N	—
Aberayron	viii. 53	N	—
Aberystwyth	viii. 53	N	—
	vii. 57	N	—
Borth	iv. 52	N	—
Barmouth	viii. 53	N	—
	v. 57	O	—
Mochras	viii. 53	N	—
Harlech	iv. 50	N	—
	iv. 56	N	—
Criccieth	vi. 52	N	—
	iii. 53	N	—
	iii. 54	R	E.W.K.J.
	v. 56	R	—
Afon Wen	v. 53	N	—
Pwllheli	v. 53	N	—
	viii. 55	O	E.W.K.J.
Llanbedrog	v. 53	N	—
	viii. 55	O–R	—
Abersoch	v. 53	N	—
	viii. 55	R	—
Trwyn Cilan	viii. 55	N	—
Hell's Mouth	viii. 55	N	—
Porth Oer	viii. 55	N	—
Nevin and Porth Dinlleyn	iv. 50	N	—
	viii. 55	C	None on exposed reefs, confined to bays
Trevor	v. 53	N	—
	viii. 55	F	—
Clynnog	iv. 50	N	—
	xii. 54	O	—
Llandwrog	xi. 51	N	—
	v. 55	O	—
Caernarvon	iv. 50	N	—
	x. 51	R	—
	xii. 52	N	—
	v. 53	N	—
	ix. 53	R	—
	viii. 55	F	—
Port Dinorwic	ii. 52	N	—
	v. 53	N	—
	v. 54	F	—
	vii. 55	F	—
Bangor	xi. 51	N	—
	ii. 52	N	—
	iii. 53	O–R	—
	xi. 53	C	Common, tubular bridge
	iii. 54	C	—
	iii. 55	A	—

Place	Date	Density	Notes
Llanddwyn	iv. 52	N	—
	v. 55	F	On mussel bed
Aberffraw	x. 51	N	—
	iii. 53	N	—
	xi. 53	N	—
	vi. 54	O	—
	viii. 55	F–C	—
Rhosneigr	iii. 52	N	—
	iii. 55	O	—
	viii. 55	F	—
Rhoscolyn	viii. 53	N	—
Porth Dafarch	v. 53	N	—
	viii. 55	N	—
Holyhead	iv. 50	R	—
	xi. 51	N	—
	v. 53	N	—
	viii. 55	F–C	Rather more common in inner harbour where there are fewer *B. balanoides*. Very common in inland sea
Church Bay	v. 53	N	—
	viii. 55	R	Little suitable substratum
Cemaes Bay	iv. 50	N	—
	vii. 53	N	—
	viii. 55	C	On walls, inner harbour
Bull Bay	iv. 50	N	—
	ii. 53	N	—
	vi. 53	N	—
	vi. 54	N	—
	viii. 55	F	—
Amlwch	iv. 50	N	—
	i. 53	N	—
	iii. 54	O	In submarine tunnel
Point Lynas Bay	xii. 52	N	—
	vi. 54	F	Occasional specimens on headland
Llys Dulas	ix. 52	N	—
Moelfre	xii. 52	O	—
Benllech	xi. 51	N	—
	vi. 52	N	—
	xii. 52	F	All of small size
	vi. 55	C	—
Penmon	iv. 52	N	—
Black Rock	viii. 52	O	—
	iv. 53	F	—
	iii. 54	C	—
Menai Bridge	vii. 51	N	—
	iv. 52	R	—
	iv. 53	O	Young spat frequent on pier, few elsewhere
	xii. 54	A	—
	vii. 55	A	—
Llanfairfechan	x. 51	N	—
	vi. 52	R	—
	xi. 53	F–C	—
	xii. 54	A	—
Conway	vii. 51	R	One specimen on a boat. E.W.K.J.
	x. 51	R	—
	ix. 52	O	On mussels in River Conway
	vi. 53	C	Settling in fair numbers, 2–3/cm^2
	vii. 55	A	—
Llandudno	x. 47	N	P.N.J.C.
	iii. 50	R	—
	xi. 51	R–O	—
	vii. 52	F–O	—
	x. 54	C–A	Less common towards Orme's Head
Colwyn Bay	ix. 47	N	—
	iii. 50	N	—
	iii. 51	N	—
	xi. 51	F	0·04/cm^2 on pier piles, where it is most abundant
	vii. 52	C	Adults 0·3/cm^2, spat settling heavily
	ix. 52	A	—
Abergele	xi. 51	A	—
Rhyl	ix. 47	N	—
	v. 50	N	—
	xi. 51	A	Common in river, abundant on piles of pier

APPENDIX (*cont.*)

Place	Date	Density	Notes
Rhyl	vi. 53	A	—
Mostyn	xi. 51	O	—
	vi. 53	F	—
Greenfields	vi. 53	A	—
Connahs Quay	vi. 53	A	Survived high cyanide concentration during fish mortality of 1953, except in immediate vicinity of effluent
Heswall	xi. 51	C	—
West Kirby	v. 50	N	—
	xi. 51	C	—
	vi. 53	C A	—
New Brighton	x. 47	N	P.N.J.C.
	v. 50	A	—
	xi. 51	A	—
	vi. 53	A	—
Liverpool	x. 47	N	P.N.J.C.
	v. 50	O	—
	xi. 51	C	—
Point of Air	ix. 55	N	Very unsuitable area, shingle scour
Ramsey	i. 52	R	A.J.S.
	ii. 53	N	A.J.S.
	ix. 55	F	A few 1–1½ years old
Laxey	ix. 55	O	Rare except at H.W near stream
Douglas	ix. 55	R	Two large individuals only
Castletown	ix. 55	N	—
Port Erin	ix. 55	N	—
Peel	ix. 55	N	—
Lytham St Anne's	x. 47	N	P.N.J.C.
	v. 50	O	—
Blackpool	v. 50	C	—
	ix. 53	A	—
Rossal	x. 47	N	P.N.J.C.
	v. 50	F	—
	x. 53	C	Heavy scour reduces numbers
River Wyre, Fleetwood	v. 50	O	—
Morecambe	vi. 50	A	Growing on parieties and valves of existing *B. balanoides* population
Grange-over-Sands	viii. 52	A	—
Bardsea	vii. 53	A	—
Barrow, Walney Channel	vi. 50	O	—
Barrow, Walney Island	vi. 50	N	--
	vii. 53	F–C	—
Millom	viii. 52	F	—
Ravenglass	vi. 50	O	On railway bridge, only 1 or 2 specimens
	viii. 52	C	On mussel beds, less common than *B. balanoides*
	vii. 53	C–A	Nearly as common as *B. balanoides*
	viii. 55	A	*Elminius* three times as common as *B. balanoides*. Spatfall 1·5/cm^2
Seascale	vi. 50	N	—
	viii. 52	F–C	About 0·1/cm^2, patchy, much less common than *B. balanoides*
	vii. 53	C	About 0·25/cm^2 in groups
	viii. 55	A	About 1·5/cm^2, equal to *B. balanoides*
St Bees	vi. 50	N	—
	viii. 52	O–R	—
St Bees	vii. 53	C	As high as 0·2/cm^2 in patches
	viii. 55	C	Adults 0·06/cm^2, spat 1/cm^2
Whitehaven	vi. 50	N	—
	vii. 53	F–O	Up to 0·04/cm^2
	viii. 55	C–A	Abundant on loose stones in harbour
Harrington	vi. 50	N	—
	vii. 53	R	—
Workington	viii. 52	N	Much scour, not very suitable
Maryport	vi. 50	N	—
	viii. 52	N	—
	viii. 55	C–A	—
River Nith, Overton Merse	iii. 51	R	Found at H.W. where only suitable stones exist. P.N.J.C.
	viii. 55	F	—
Sutherness Point	x. 50	R	—
	viii. 55	A	Spatfall 25/cm^2
Rockliffe, Rough Firth	viii. 55	A	—
Port Mary	viii. 55	O	Mainly at L.W. under stones
Manxman's Lake	viii. 55	F	—
Kircudbright Bridge	viii. 55	C	—
Fleet Bay	ix. 53	N	—
	viii. 55	C–A	All rather small, spatfall 10/cm^2
Ravenshall Rocks	viii. 52	N	—
	ix. 53	N	—
	viii. 55	O	—
Creetown	viii. 52	N	—
	viii. 55	C	Much less common than *B. balanoides*
Garliestown	viii. 55	O	No spatfall seen
Isle of Whithorn	viii. 55	O	—
Monreith Bay	viii. 55	N	—
Luce Bay, Port William	ix. 53	N	—
	viii. 55	N	—
Auchenmalg Bay	ix. 53	N	—
	viii. 55	N	—
Sandhead	ix. 53	N	—
	viii. 55	N	—
Ardwell	ix. 53	N	—
	viii. 55	N	—
Drummore	ix. 53	N	—
	viii. 55	R	—
Port Patrick	i. 50	N	—
Corsewall Pt	ix. 53	N	—
Stranraer, Loch Ryan	i. 50	O	—
	ix. 53.	O–R	All old specimens
	ix. 55	O–R	No change
Kirkcolm	ix. 53	R	—
Ballantrae	i. 50	N	—
	ix. 53	N	—
Lendalfoot	ix. 53	N	—
Girvan	ix. 53	N	—
Ayr	i. 50	N	—
Troon	i. 50	N	—
	x. 53	N	—
Ardrossan Saltcoats	i. 50	N	—
	x. 53	N	—
West Kilbride	x. 50	N	—
Fairlie	x. 50	N	—
Largs	x. 50	N	—
	x. 53	N	—
Dumbarton	x. 50	N	—
Millport, Isle of Cumbrae	x. 50	N	—
	1955	R	J.H.C.

Observers: J.H.B., Mr J. H. Barrett; M.W.H.B., Mr M. W. H. Bishop; H.O.B., Dr H. O. Bull; P.N.J.C., Dr P. N. J. Chipperfield; H.A.C., Dr H. A. Cole; J.H.C., Dr J. H. Connell; M.W.H.D., Mr M. W. H. Dowell; C.M.H., Miss C. M. Harrison; E.W.K.J., Prof. E. W. Knight-Jones; A.H.N.M., Mr A. H. N. Molesworth; E.N. Mr E. Norris; J.H.O., the late Prof. J. H. Orton; A.J.S., Dr A. J. Southward; E.A.S., Prof. E. A. Spaul; H.G.S., Dr H. G. Stubbings; G.D.W., Dr G. D. Waugh; D.P.W., Dr D.P. Wilson.

Careful searches have also been carried out on west, north and east coasts of Scotland, and on the coasts of Ireland, especially the ports Belfast, Dublin, Larne and Rosslare. Up to 1953 no *Elminius* was found north of Loch Ryan in Scotland, nor anywhere in Ireland.

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SURVIVAL OF ANAEROBIC PERIODS BY TWO INTERTIDAL POLYCHAETES, *ARENICOLA MARINA* (L.) AND *OWENIA FUSIFORMIS* DELLE CHIAJE

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Borden (1931) found that the lugworm *Arenicola marina* L. did not increase its rate of oxygen consumption after short (2 h) periods under anaerobic conditions, and concluded that there was no evidence that these animals could go into debt for oxygen after the supply of oxygen in the blood had been used. She calculated that there was sufficient oxygen in the blood to last the worm about 1 h, calculating that the oxygen capacity per gramme worm was 0·037 ml., and the oxygen consumption per gramme hour was 0·031 ml. As the period during the experiment when oxygen was excluded from the animals was 2 h, anaerobic respiration would have continued for about 1 h, which should have been sufficient to necessitate an easily measurable increase in the oxygen consumption on return to normal conditions if a debt for oxygen had been produced. In vertebrates, and some invertebrates, such a debt would be incurred by glycolysis, the lactic acid produced being reoxydized to glycogen in the presence of free oxygen, and thus producing an initially increased oxygen consumption on return to aerobic conditions.

The absence of an oxygen debt after a period of anaerobic conditions may be due to one or more of several causes. During an anaerobic period, a poikilothermic animal may (1) reduce its metabolic rate to a very low level, (2) metabolize glycogen to an end-product other than lactic acid, (3) excrete the lactic acid produced, or (4) utilize protein or oil rather than glycogen as an energy source. In polychaetes there is also the possibility that lactic acid could be accumulated in the coelomic fluid and only slowly reoxidized. In all these instances no marked increase in oxygen consumption would be expected on return to normal aerobic conditions.

The method of surviving anaerobic periods by polychaetes is interesting in view of the regular or occasional subjection of some species to such conditions, especially in the intertidal zone, and of their ability to survive such conditions in the laboratory. Borden (1931) found *Arenicola* to be unaffected by short periods, and Hecht (1932) found that lugworms would in fact survive for as long as 9 days without oxygen. *Owenia fusiformis* Delle Chiaje is even more resistant; von Brand (1927) found that this species could survive for 21 days under strictly anaerobic conditions. Packard (1905) found *Amphitrite* and

Nereis to survive a day; Jacubowa & Malm (1931) investigated the survival of several species of polychaetes, and found them to survive for periods of from 1 to 10 days. Neither Borden (1931) nor Hecht (1932) made glycogen or lactic acid determinations on *Arenicola*, but von Brand (1927) found 0·38% of the wet weight in the lugworm was glycogen, and 5% in *Owenia*; in an earlier paper (Dales, 1957*a*) I found 1–2% in *Arenicola*, and in the present work 2% in *Owenia*.

The long survival time of *Owenia* coupled with the high glycogen content reported by von Brand (1927) suggests that survival of long anaerobic periods may be made possible by metabolism of glycogen. Also the knowledge that *Arenicola* can survive anaerobic periods much longer than could be endured solely by utilization of oxygen in the blood, yet does not show an increased oxygen consumption on return to normal aerated conditions, indicates that if glycogen breakdown does occur, this either does not lead solely or mainly to lactic acid as in vertebrate glycolysis, or the lactic acid is wholly excreted.

Consequently, the glycogen content of various parts of the body of *Arenicola* and of *Owenia* has been measured in worms under normal aerobic, and under varying periods of anaerobic conditions. The oil content, lactate and pyruvate content have also been estimated, and the distribution of glycogen and oil studied histochemically. The results are discussed in relation to the actual conditions with which these worms may contend in nature. The *Arenicola* were mostly collected at Chalkwell, Essex, rather small worms being used for convenience in the experiments, but some duplicate experiments were done on Plymouth worms which were much larger. The *Owenia* studied were collected from Tor Abbey sands, Devon, and from near St Mawes in Cornwall.

Much of this work was done at the Plymouth Laboratory and it is a pleasure to acknowledge the continued interest and help shown by the Director and Staff. I also wish to thank Miss M. Weir for doing much of the histological work, and Dr J. Green and Mr R. F. H. Freeman for performing Winkler oxygen determinations on the water in the experimental vessels. I am grateful for a grant awarded by the University of London Central Research Fund, and for the use of the London Table at Plymouth; and to Prof. G. P. Wells, Dr E. D. S. Corner, and Dr J. Green for reading the typescript and for their helpful criticism.

METHODS

Worms were placed in a 'Thermos' flask with ice immediately on collection, and dealt with as soon as possible on return to the laboratory, always within 3–4 h. The glycogen content of animals described here as 'fresh', are those so treated and weighed immediately on reaching the laboratory.

In the experiments the weights of all the *Arenicola* refers to whole animals minus tails, coelomic fluid and gut. The weights of the *Owenia* refers to complete animals including gut and coelomic fluid. In both cases wet weights were obtained after drying on filter-paper; although the *Owenia* were small, the body is smooth and cylindrical and the animal is easily and quickly dried.

The experimental animals were placed in 500 ml. conical flasks half filled with sea water, with three small *Arenicola* or twenty *Owenia* in each. The *Owenia* were not removed from their tubes but these were seen to be clean before being used; the *Arenicola* were washed before use in an experiment. Experiments were performed in a constant temperature room at 18° C. To obtain anaerobic conditions a stream of nitrogen from a cylinder was passed through alkaline pyrogallol wash-bottles into a trap from which a number of closable jets protruded, each of which could be connected to an experimental flask. A stream of nitrogen was maintained through the water until the oxygen content was negligible before the animals were introduced. Then a very slow but continuous stream was maintained for the duration of the experiment, the gas escaping through a bunsen valve in the stopper closing the flask. In a few instances the oxygen contents of the water during and at the end of an experiment was checked with Winkler's reagents, and found to be no more than would be expected in the reagents. The gas was therefore not passed over heated copper as well as through alkaline pyrogallol as recommended by von Brand (1946) as the cylinders of nitrogen used were sufficiently pure. In all the experiments the animals were fasting.

Glycogen was estimated by hydrolysis to glucose which was then determined by Hagedorn & Jensen's method (1923) as described in a previous paper (Dales, 1957*a*). Lactate was estimated by the method of Barker & Summerson (1941), the tissue being fixed by dropping the rapidly weighed tissue into ice cold 10% trichloroacetic acid. Pyruvate was estimated by Lu's method (1939) using 2:4-dinitrophenlyhydrazine and extracting with ethyl acetate. The final colour in both lactate and pyruvate determinations was measured with a 'Unicam' S.P. 500 spectrophotometer, the lactate at 570 mμ, pyruvate at 550 mμ, against standards of known purity.

Total oil was measured gravimetrically by the method of Sperry (Glick, 1955). The tissue was disintegrated in a Griffeth pattern tissue disintegrator and dried with acetone which was then blown off in a current of nitrogen, the sample being left to dry in a nitrogen-filled desiccator. The dried tissue was weighed and then extracted with 2:1, $CHCl_3$:MeOH in the cold, the extract poured off and the tissue re-extracted with three successive portions of solvent. The extract was poured through fat-free filter-paper into a flat-bottomed tube, and the paper rinsed through. The combined extract was then stood in a large volume of distilled water overnight, and the aqueous layer then removed (without flocculent interface) by suction. The solution was then transferred to a small flask and evaporated to dryness *in vacuo*, the oil redissolved in $CHCl_3$ and transferred to a small weighing bottle weighing rather less than 1 g. The $CHCl_3$ was evaporated and the sample left in a nitrogen-filled desiccator until ready for weighing. The weight of the oil was determined by difference after redissolving away the oil with $CHCl_3$. An aperiodic microchemical balance with a sensitivity of 0·1 mg was used.

Distribution of glycogen and oil was studied by the methods previously described (Dales 1957*a*). Glycogen was stained by Best's method after embedding in ester wax (Smyth & Hopkins, 1948), and oil with acetylated sudan black B (Casselman, 1954) in propylene glycol (Chiffelle & Putt, 1951), after formal-calcium fixation (Baker, 1946), frozen sections being cut from gelatin embedded tissue.

RESULTS

Arenicola marina

The results of estimations of glycogen content of the body wall of animals under various conditions are summarized in Table 1. It will be seen that the glycogen content falls under conditions of fasting when aerated, but that much more glycogen is used under anaerobic conditions. Although the glycogen content of the gut was found to be much lower than that of the body wall, the concentration in 'fresh' animals and those under anaerobic conditions was also measured. The fall is not statistically significant. To test whether the glycogen content would rise in fasting worms after an anaerobic period (as in vertebrate glycolysis) the glycogen content was measured at the end of a 40 h period under anaerobic conditions and after a further 6 h under fresh aerated sea water. The concentration did not, however, approach that of the fresh control worms.

TABLE 1. *ARENICOLA:* GLYCOGEN IN THE BODY WALL AND GUT

	No. of estimations	Mean (wet) weight (g)	Extreme weights	Mean total glycogen (mg)	Standard deviation total glycogen	Mean concentration glycogen (mg/g)	Standard deviation glycogen concentration
Body wall							
'Fresh'	40	0·398	0·116–0·975	6·80	3·85	16·82	4·57
Fasting, 40 h aerated	30	0·263	0·088–0·819	3·42	2·51	11·95	3·51
Fasting, 40 h under anaerobic conditions	19	0·310	0·093–0·655	2·55	2·01	7·03	3·90
Fasting, 40 h under anaerobic conditions; aerated 6 h	12	0·219	0·097–0·305	2·11	1·17	9·85	4·60
Gut							
'Fresh'	15	0·151	0·069–0·296	1·27	0·63	8·32	2·86
Fasting, 40 h under anaerobic conditions	15	0·078	0·028–0·138	0·52	0·81	7·11	2·54

Oil content of the body wall in 'fresh' worms and those subjected to anaerobic conditions for 48 h was also measured (Table 2), and there was found to be no change.

Concentration of lactate in the body wall was not significantly different in 'fresh' worms, and those subjected to anaerobic conditions for 42 h, aerated controls or those returned for 6 h to fresh aerated sea water (Table 3). As any lactate secreted might be broken down by bacteria in the medium, in order to test whether any secretion of lactate occurs under anaerobic conditions, one series of experiments was done under the same conditions as before, but using specially cleaned glassware and boiled and autoclaved sea water to which 100 μg ml. of streptomycin sulphate had been added. A detectable, but negligible amount of lactate was found in the medium, after 40 h under anaerobic conditions with three worms (total mean weight 10 g) in 250 ml. sea water (Table 3).

Pyruvate was not detectable in the tissues of worms after 48 h under anaerobic conditions.

TABLE 2. *ARENICOLA*: OIL CONTENT OF BODY WALL

	No. of estimations	Mean (dry) weight (g)	Extreme weights	Mean total oil (mg)	Standard deviation total oil	Mean oil concentration (mg/g)	Standard deviation oil concentration
Group A							
'Fresh'	15	0·3380	0·2152–0·7115	17·1	7·0	50·20	5·59
After 48 h under anaerobic conditions	14	0·3877	0·2174–0·7694	18·8	6·7	49·20	6·52
Group B							
'Fresh'	13	0·1775	0·1132–0·2968	7·6	1·8	43·90	5·55
After 48 h under anaerobic conditions	11	0·0951	0·0658–0·1420	4·2	1·3	44·46	6·52

Group A = Plymouth worms (August, large); B = Chalkwell worms (October, small).

TABLE 3. *ARENICOLA*: LACTIC ACID IN THE BODY WALL

	'Fresh'	Fasting, aerated 42 h	Fasting, under anaerobic conditions 42 h	Fasting, under anaerobic conditions 42 h; aerated 6 h	Concentration in the external medium after 40 h under anaerobic conditions
No. of estimates	9	12	15	10	10
Mean concentration (mg/g)	0·297	0·338	0·365	0·362	0·007
Standard deviation of mean	0·164	0·180	0·165	0·215	0·0036

Owenia fusiformis

The glycogen content of whole 'fresh' *Owenia* and those that had been subjected for 5 days and 9 days to anaerobic conditions are shown in Table 4. There appears to be no fall in glycogen content. Consequently, no measurements of lactate were made. Estimations of oil content (Table 5) of 'fresh' worms and those subjected to 5 days of anaerobic conditions showed no significant change in concentration. There was no significant fall in weight after 9 days under anaerobic conditions, nor did the green pigment in the intestine (Dales, 1957*b*) appear to decrease in quantity.

DISCUSSION

It is quite clear that in *Arenicola* metabolism of glycogen under anaerobic conditions leads to products other than lactic acid. This is consistent with the findings of von Brand (1946), who concluded that invertebrates generally metabolized glycogen to a mixture of acids; which acids are produced by *Arenicola* has not been determined. Thus, while an oxygen debt may be avoided, the resources may be wasted if normal activity is continued. Vertebrate glycolysis may be more economical of material, but this incurs an oxygen debt which can only be cleared in a short time by an efficient respiratory mechanism. Even if true glycolysis existed in the lugworm, it might be that the less efficient respiratory mechanism would be unable to clear the debt within a reasonable period.

TABLE 4. *OWENIA*: GLYCOGEN CONTENT OF WHOLE WORMS

	No. of estimations	Mean (wet) weight (g)	Extreme weights	Mean total glycogen (mg)	Standard deviation mean total glycogen	Mean concentration glycogen (mg/g)	Standard deviation concentration
'Fresh'	17	0·059	0·027–0·104	1·215	0·53	21·0	8·8
After 10 days in laboratory (aerated)	10	0·055	0·033–0·073	1·064	0·30	19·9	4·5
After 5 days under anaerobic conditions	32	0·059	0·023–0·096	0·978	0·41	20·7	7·9
After 9 days under anaerobic conditions	34	0·053	0·025–0·089	1·048	0·45	20·2	6·4

TABLE 5. *OWENIA*: OIL CONTENT OF WHOLE WORMS

	No. of estimations	Mean (dry) weight* (g)	Extreme weights*	Mean total oil (mg)	Standard deviation total oil	Concentration of oil (mg/g)	Standard deviation of concentration
'Fresh'	10	0·1961	0·1647–0·2337	11·6	1·54	59·27	8·28
After 5 days under anaerobic conditions	12	0·1887	0·1370–0·2245	12·2	1·84	64·30	3·45

* Twenty worms weighed together.

It also seems likely that in nature anaerobic periods would be short, and the glycogen used could easily be replenished. Intertidally, the burrows are probably rarely uncovered for more than 9 h, and commonly for much less, although Hecht (1932) found some worms in a situation covered only by spring tides. Presumably aerial respiration (the trapping of bubbles of air in the burrow, described by van Dam, 1938, Wells, 1945, 1949) would play an important part in the lives of these worms, and anaerobic periods are more likely to be encountered where a layer of stagnant water remains over the burrow openings. But even if exposed for 9 h to completely anaerobic conditions, the glycogen concentration of the body wall would fall only by 2 mg/g, and the worm would lose 11 % of its total glycogen. Probably lugworms are rarely subjected to such long periods, and even when they are, there is every indication that the activity of the animal would be reduced. In the experiments the worms became rather quiescent after some time without oxygen, though still showing spontaneous body-wall contractions and bursts of activity. This agrees with Wells' findings (1949). Dr J. D. Jones found no significant difference in lactate content in worms dug at the beginning and end of a 5 h intertidal period (private communication).

It is already well known (Barcroft & Barcroft, 1924; Borden, 1931; Wolvekamp & Vreede, 1941; Wells, 1949; Jones, 1954) that the oxygen dissociation curve of *Arenicola* haemoglobin shows that oxygen may be taken up at low outside concentrations, lower than usually occurs in an exposed burrow after 5 h exposure which was higher than that of the interstitial water (Jones, 1954) perhaps owing to aerial respiration by the worm. Wolvekamp & Vreede (1941) have criticized Barcroft & Barcroft's original idea (1924) that the oxygen in the blood acted as a store for use by the animal when the burrow was uncovered by the tide, but maintained that it was of use during resting periods in the irrigation of the burrow. More recent writers (Wells, 1949; Jones, 1954) have also inclined to minimize the usefulness of the oxygen in the blood for surviving anaerobic periods. On the other hand, in view of Wells' (1949) observations on aerial respiration and the characteristics of the haemoglobin, do lugworms often have to contend with long anaerobic periods? Linke (1939) found the temperature of the surface water on an *Arenicola* beach to rise as high as 26·2° C, and to 21·3° C at 10 cm depth; Thamdrup (1935) found that the oxygen consumption of *Arenicola* falls above 20° C, and Wells (1949) that under such conditions the normal irrigation of the burrow would cease. Wells found that worms would withstand 7 h in his glass apparatus when this was closed and which, at the time of closure would contain about 0·2 c.c. oxygen. The behaviour was modified completely under such conditions, the worm making only occasional 'testing' movements, though responding at once when a fresh supply of oxygenated water became available. This suggests that, even though glycogen is used under anaerobic conditions, worms conserve their energies by becoming quiescent.

This is certainly so in *Owenia*, in which there was no significant fall in glycogen concentration after 9 days under anaerobic conditions. After a few hours the worm became quiescent, and after some days, coiled up tightly in the middle of the tube and showed no sign of activity. Nothing is known about the characteristics of the haemoglobin in this species. The reason why *Owenia* does not use any of its glycogen, whereas *Arenicola* does, though reducing its activity, may also be related to the site of the glycogen deposits which is mainly in the body wall in the lugworm, but in the coelomic cells in *Owenia*. It is unlikely that these cells constitute reserves to be drawn upon during periods of inanition (Dales, 1957*a*); further work on their function is in progress. Again, while *Arenicola* shows bursts of activity under anaerobic conditions, *Owenia* does not.

Von Brand (1946) found that the quotient of aerobic: anaerobic glycogen consumption was low, although he found a relatively high glycogen content, commenting: 'Whether this indicates an exceptionally great reduction of metabolic rate under anaerobic conditions, or a more pronounced participation of fat or protein in anaerobic degradation processes, or whether it is due to fermentative processes in aerobic conditions has not been established.' The rather large discrepancy between von Brand's figures (1927) for glycogen content of *Owenia*, and those obtained here, may perhaps be accounted for by the difference in weight, his worms were six times heavier, weighing 300 mg each. Von Brand (1927) found that, under anaerobic conditions, 0·23 g glycogen 100 g worm/24 h was consumed. If consumption continued at this rate, all would have been used in 21 days, the survival time von Brand found. *Owenia* from Tor Abbey sands being much smaller may have relatively less in the body wall, so that the consumption of this could not be detected in the presence of the large quantities in the coelomic cells. This ability to reduce activity may well have adaptive significance as these worms may temporarily be buried by the shifting sand they inhabit.

The ability to reduce the metabolic rate may be general in other polychaetes. There seems to be no correlation between the survival time of different species under anaerobic conditions (von Brand, 1946) and the glycogen content (von Brand, 1927). Indeed those species with the highest values for glycogen are the more specialized tube dwellers, which are often provided with well-developed coelomic cells in which most of the body glycogen is stored, and which seem least likely to encounter anaerobic conditions in nature.

SUMMARY

Measurements of glycogen in the body wall of *Arenicola* indicate that glycogen is consumed during anaerobic conditions. Estimations of lactate and pyruvate show that neither is accumulated, accounting for the absence of an oxygen debt previously found by other workers, and suggesting that glycogen breakdown leads to other acids. In *Owenia* most of the glycogen is stored in coelomic cells and these deposits are not drawn upon during anaerobic periods, yet this species can survive long periods without oxygen, apparently by becoming quiescent. Oil content in both species has also been measured, and was found not to fall under anaerobic conditions. It is suggested that survival of anaerobic periods may be mainly due to an ability to suspend normal activity.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

Blaschko, H. & Hope, D. B., 1957. Observations on the distribution of amine oxidase in invertebrates. *Arch. Biochem. Biophys.*, Vol. 69, pp. 10–15.

The tissues of a number of marine invertebrates have been examined for the presence of the enzyme amine oxidase. No enzymic activity was found in three species of tunicates examined. Tryptamine and β-phenylethylamine were oxidized by extracts of gut of a polychaete, *Chaetopterus variopedatus*. Enzymic activity was found to be present in many, but not all, of the species of molluscs examined; of particular interest is the finding that the digestive gland and the anterior retractor muscle of the byssus in *Mytilus edulis* contain the enzyme; the latter muscle is known to be highly sensitive to 5-hydroxytryptamine. Among the echinoderms, all asteroids and echinoids had amine oxidase activity but no activity was detected in holothuroids. The presence of the enzyme in echinoderms suggests that biogenic amines, hitherto not detected, occur in these animals. H.B.

Dartnall, H. J. A., 1957. The spectral variation of the photosensitivities of some visual pigments. *Symposium on visual problems of colour, held at Nat. Phys. Lab. Teddington, Middx., from 23rd to 25th September* 1957, Paper No. 3.

The photosensitive retinal pigments of the tench (*Tinca tinca*), the carp (*Cyprinus carpio*), the gurnard (*Trigla cuculus*) and the conger eel (*Conger conger*) were extracted by means of 2% digitonin solutions.

It was found that the bleaching kinetics of these four pigments could be described by the same equation as that previously developed for the well-known rhodopsin or visual purple of the frog. The photosensitivities of the pigments were measured as ratios relative to the frog pigment over the wavelength range 440–580 mμ.

The absolute values of the photosensitivities of the four pigments were obtained by multiplying the ratios by the known values for the absolute photosensitivities of the frog pigment. H.J.A.D.

Hanson, Jean & Lowy, J., 1957. Structure of smooth muscles. *Nature, Lond.*, Vol. 180, pp. 906–9.

The detailed structure of smooth muscles from various invertebrate (particularly molluscan) and vertebrate animals was investigated by electron microscopy and phase-contrast light microscopy. It was found that there are several very different types of fibres: there is no 'typical' smooth muscle. The dimensions and structure of the protein filaments are highly variable. They may be randomly arranged in the fibre or closely and regularly packed into distinct myofibrils. In the muscles of squid (*Loligo*) the myofibrils are helically arranged and are composed of two kinds of filaments which are cross-linked to each other and closely resemble those in the anisotropic bands of cross-striated myofibrils; but the smooth fibrils have this construction along the whole of their length. The mechanism of contraction is probably the same in these particular smooth muscles as in cross-striated muscles. Other muscles have neither cross-striations nor a double array of filaments. In vertebrate smooth muscles the filaments are all very thin and look alike. In the white (slow) part of lamellibranch adductors the 'filaments' are very large and appear to be composed of ribbon-shaped subfilaments with the elaborate 'paramyosin' structure. J.L.

Hobson, G. E. & Rees, K. R., 1957. The annelid phosphokinases. *Biochem. J.*, Vol. 65, pp. 305–6.

Following the isolation of taurocyamine and glycocyamine from a number of annelid worms, it was suggested that these guanidine bases, when phosphorylated on the terminal amino group functioned as 'phosphagens'. We have confirmed that fresh body-wall muscles from a number of polychaete worms very often contain a mixture of glycocyamine phosphate with either taurocyamine or creatine phosphate. Furthermore, we have shown the presence in the same tissues of the appropriate phosphokinase enzyme systems, capable of building up the phosphates from the guanidine and adenosine triphosphate.

The existence of these active transphosphorylation systems is held as evidence for the products serving a new but typical 'phosphagens' *in vivo*, for certain of the annelids. Their distribution, however, appears to be completely arbitrary. K.R.R.

Jones, W. C., 1957. The contractility and healing behaviour of pieces of *Leucosolenia complicata*. *Quart. J. micr. Sci.*, Vol. 98, pp. 203–17.

Isolated pieces of the body wall, when left in sea water or in $MgCl_2$: sea water, undergo a process of reorganization whereby the tubular form of the olynthus is regained. The piece first curls with the choanoderm on the inner surface, and a membrane then spreads from the four edges, closing off a new spongocoel. The membrane consists of the two surface epithelia with a thin layer of mesogloea in between. Its spread is accompanied by a shrinkage of the area of the original piece.

When the internal epithelium has been brushed away, the pieces shrink rapidly and curl longitudinally towards the dermal side.

The experiments indicate that both epithelia are contractile. There is evidence that the porocytes are responsible for the contractility of the internal epithelium and are interconnected beneath the bases of the choanocytes. W.C.J.

Nicol, J. A. C., 1957. Observations on photophores and luminescence in the teleost *Porichthys*. *Quart. J. micr. Sci.*, Vol. 98, pp. 179–88.

Histology and physiology of the photophores of an inshore teleost, *Porichthys myriaster*, are described. Photophores lie in the dermis: each consists of a lens, photogenic and reflecting layers, and is supplied by a nerve. Luminescence in the living fish was evoked by electrical stimulation of the spinal cord, and by injection of adrenaline. Latency, following stimulation of the cord, was 7–10 sec; after intracardiac injection of adrenaline, 2 min. Electrical stimulation of the cord produced luminescence after arrest of the circulation. It is suggested that the photophores of *Porichthys* are under control of the sympathetic nervous system. J.A.C.N.

Ross, D. M., 1957. Quick and slow contractions in the isolated sphincter of the sea anemone, *Calliactis parasitica*. *J. exp. Biol.*, Vol. 34, pp. 11–28.

This paper deals with the physiology of isolated marginal rings of the sea anemone, *Calliactis parasitica*, a preparation containing the marginal sphincter muscle which is responsible for the powerful closing movement of this animal. Besides this quick contraction, so well known from the work of Pantin as a unique example of a facilitated response, the preparation shows slow contractions in response to stimuli at lower frequencies. On account of the relative absence of spontaneous movements in this preparation it was possible to obtain a good deal of information about relationships between the size and latent period of the slow contractions obtained and the number and frequency of stimuli applied. There is a threshold number of stimuli at each frequency which causes only a tiny response and there is an optimal frequency at which the biggest responses are obtained with fewest stimuli. In considering the possible causes of 'fast' and 'slow' activities in the margin of *Calliactis*, the general evidence is in favour of an explanation which imagines muscle fibres of the same type which can contract in two different ways according to the number and frequency of impulses reaching them, and possibly according to the route by which the impulses travel.

D.M.R.

STOTT, F. C., 1957. Observations on the food canal and associated structures in the holothurian *Holothuria forskali* delle Chiaje. *Proc. zool. Soc. Lond.*, Vol. 129, pp. 129–36.

A simpler nomenclature is suggested for the parts of the holothurian gut. The rete mirabile of *Holothuria forskali* showed three regions, namely, a relatively unpigmented region attached to the dorsal edge of the stomach which connected by a single strand to a yellowish brown region alongside the intestine which had a brownish black edge to it filled with melanin granules. Feeding with saccharated iron carbonate showed that some amoebocytic ingestion took place in the stomach and intestine and clumps of amoebocytes containing iron appeared in the haemal canals, lumen of the rete mirabile and in the walls of the respiratory trees, where it was probably egested. Injection of trypan blue into the body cavity resulted in its phagocytosis by the agranulocytes and their clumping together with the granulocytes on the respiratory trees, gonads and intestine. Migration into the lumen of the latter was observed. Melanin granules were identified within granulocytes in the lumen and walls of the rete mirabile as well as in the cells of the rete wall.

F.C.S.

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OBSERVATIONS ON LUMINESCENCE IN *NOCTILUCA*

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(Text-figs. 1–9)

Noctiluca miliaris Suriray is a well-known luminescent organism, and its light emission has been studied repeatedly. Hitherto, however, no attempt has been made to record the luminescent flashes of this species, either under normal conditions, or after experimental treatment. With the sensitive photoelectric recording methods now available it has become possible to subject the luminescent flashes of *Noctiluca* to experimental analysis, and with this end in view the present study of *Noctiluca* was undertaken.

Noctiluca is a dinoflagellate approximately spherical in shape and 0·2–1·2 mm in diameter. It possesses a single stout tentacle and a small flagellum by which it executes a limited amount of slow movement. The specific gravity of *Noctiluca* is less than that of sea water so that it tends to float at the surface. The explanation of this low density is still uncertain (Davis, 1953). The interior of the cell is highly vacuolated and contains fine cytoplasmic strands which radiate outwards from the region of the nucleus to the periplast. E. N. Harvey (1952) has reviewed previous literature dealing with the luminescence of *Noctiluca*.

MATERIAL AND METHODS

During July and August 1955 there were large numbers of *Noctiluca* in the surface plankton off Plymouth, and abundant material became available for studies of luminescence. Dr M. W. Parke was successful in culturing *Noctiluca* in Erdschreiber solution. These cultures were supplied with *Isochrysis galbana* for food, and were subcultured at intervals. From them *Noctiluca* cells were removed for study when required. Further supplies of *Noctiluca* were obtained in June 1957, in the Bay of Douarneney (48° 18·5′ N., 5° 13′ W.) and the Bay of Concarneau (47° 51′ N., 3° 58′ W.) (R.V. 'Sarsia' cruise 3 of 1957).

The luminescent flashes of *Noctiluca* were detected by means of a photomultiplier tube. The photocurrent was passed through a 500 kΩ resistor, and voltage changes were amplified (DC amplifier) and recorded by means of cathode-ray oscilloscope and camera. The photomultiplier was an E.M.I. type no. 6685, with an end-window 9 mm in diameter. This photomultiplier has 14 stages and was operated at voltages up to 1800 V. The Sb-Cs cathode possesses maximal sensitivity in the violet and blue; the relative spectral sensitivity curve, as determined by the National Physical Laboratory, is shown elsewhere (Nicol, 1958*a*, p. 34, fig. 2, curve A). The specimens of *Noctiluca* were placed in a small container closely underneath the end-window of the photomultiplier and all leads were screened to eliminate a.c. interference. For the majority of records, vertical deflexions of the oscilloscope spots were photographed on moving paper; a few photographs on film were also made of single and repeat sweeps of the oscilloscope when triggered by the stimulator.

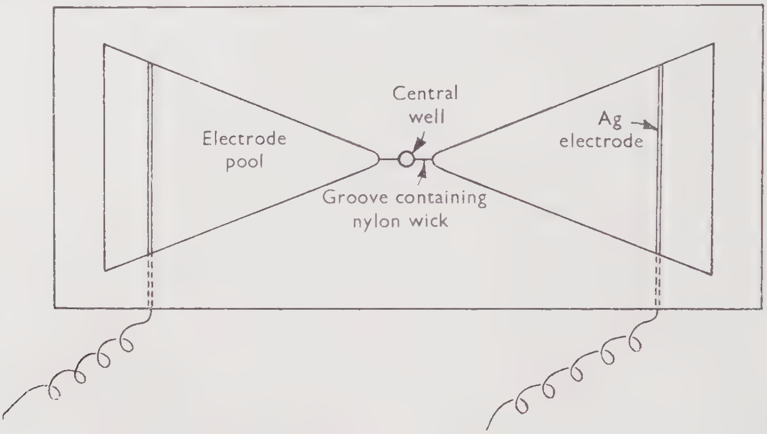

Fig. 1. Diagram of the chamber used for electrical stimulation of *Noctiluca*. Modified from Chang (1954).

For electrical stimulation *Noctiluca* was placed in a Perspex chamber, provided with two silver electrodes and shaped as in Fig. 1. The Noctiluca cell lay in the small central well, which was connected with two large lateral wells by narrow slits; the latter were filled with wet threads or agar. The whole chamber was flooded with sea water and was covered with a glass or perspex plate to prevent evaporation. Electrical stimuli consisted of condenser-discharges or square waves from electronic apparatus. Electrical stimuli were signalled on the second beam of the oscilloscope. A time signal was provided by the periodic flashing of a small lamp, controlled by a Palmer relay (this set-up was used with moving paper); or by an A.F. oscillator (used with sweeps and stationary film). Room temperatures at which the experiments were carried out varied from 18° to 19° C (shore laboratory) and 20° to 23° C (ship's laboratory).

OBSERVATIONS

MECHANICAL STIMULATION

The natural stimulus evoking luminescence in *Noctiluca* is mechanical. Placed in a vessel, Noctilucae assemble at the top, especially about the margin of the vessel. Upon agitating the container, many of the cells respond by a bright flash. Similarly, moving an object through the water, or blowing a jet of air on the surface, agitates the cells and causes them to flash. Animals moving through the water also disturb the Noctilucae, and make them luminesce. In a breffit containing a large number of Noctilucae and some *Calanus* it was observed that whenever a *Calanus* invaded the superficial Noctiluca layer, it gave rise to a bright glow in a circumscribed area, owing to agitation of a group of Noctiluca cells.

When a single cell is mechanically stimulated by agitating the container in which it lies, it is found that the intensity of the flash depends on the vigour of stimulation, a slight shake giving a faint flash, a brisk shake a stronger flash (Fig. 2*a*, *b*). At a temperature of 17° C the flash duration varied from 22 to 95 msec (a bright flash possessing a longer duration). The flash of a single cell, lasting 95 msec, had the following temporal characteristics: time to maximum, 20 msec; time to half maximum, 10 msec; decay time from maximum, 70 msec; time of half decay, 20 msec.

These figures for response characteristics are presented to give some idea of the magnitudes involved. With present recording technique they make no claim to absolute accuracy, since they depend on the amplification characteristics of the apparatus. At high amplification, threshold for measurable deflexion of the oscilloscope beam is lowered, and the response appears to start sooner, and last longer, than at low amplifications. The apparent duration of a flash, therefore, depends on the amplification-factor. (This effect has been analysed in the responses of cells stimulated electrically, and is discussed on p. 541.)

Various methods were tried to obtain more accurate regulation of mechanical stimulation than could be achieved merely by manual operation. This proved difficult because the *Noctiluca* is buffered by the pool of sea water in which it floats and because, when disturbed, it sinks and changes position. Some records were obtained by stimulating Noctilucae mechanically by means of a loud speaker. Condenser shocks, fed into the speaker, shook the dish and caused the Noctilucae to flash.

From a group of about 10 cells the following response parameters were determined for a flash lasting 120 msec (17° C). Rise to maximum occupied 43 msec; half maximum, 30 msec; decay from maximum occupied 80 msec; half decay, 20 msec. Latency from time of condenser-discharge varied from 10 to 30 msec.

Mechanical latency of the system was slightly less than 1 msec; and maximal movement occurred 23 msec after condenser-discharge. Owing to inertia of the system it is not possible to cite a value for true latency of the cell. However, the shortest interval measured from the beginning of mechanical stimulation (viz. 10 msec) shows that latency following mechanical stimulation may be of the same order of magnitude as that following electrical stimulation (viz. 9 msec).

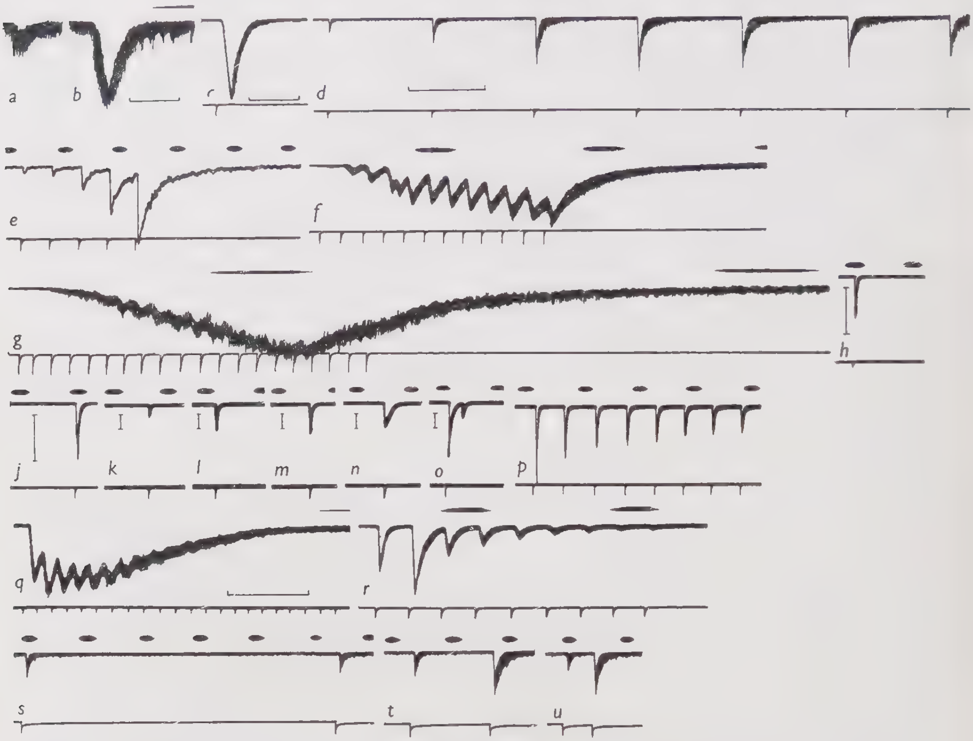

Fig. 2. Oscilloscope records of the flashes of a single Noctiluca cell. Photographs on moving paper of vertical excursions of the oscilloscope beams. *a*, *b*, flashes of a Noctiluca cell in response to mechanical stimulation (agitation of the container). *a*, strong; *b*, weak stimulus. The response is registered as a downward deflexion of the trace and is superposed on background noise. Time scale, 10 msec. Temp. 17° C. *c*, response of a Noctiluca cell to a single electrical shock. Time scale, 10 msec. Temp. 19° C. *d–g*, electrical stimulation of a Noctiluca cell at various frequencies. *d*, burst of shocks at 48/min. Time scale, 1 sec. Temp. 21° C. *e*, burst of 5 shocks at 2/sec (background noise reduced with a low band-pass filter). *f*, burst of 12 shocks at 9/sec. *g*, burst of 20 shocks at 28/sec; fusion of individual responses almost complete, long after-glow. Temp. *e*, *f*, *g*, 20° C. *h–o*, effect of increasing strength of stimulus on intensity and characteristics of the responses. *h–n*, responses of the same cell to pulses of increasing duration (shocks from 0·023, 0·5, 0·72, 1, 1·95, and 4μF condensers, respectively). Relative amplification shown to the left of each record. Note lengthening of the pulse in record *n*. *o*, double flash induced by a pulse at voltage 3 × threshold. Temp. 19° C. *p*, *q*, *r*, different patterns of responses under repetitive electrical stimulation. *p*, intervals 0·5 sec; *q*, 0·03 sec; *r*, 0·2 sec. Temp. 19° C. *s–u*, electrical stimulation of a single Noctiluca cell. Paired shocks at intervals of 5·5, 1·4 and 0·5 sec. Temp. 20·4° C. Time scale *e–p*, *r–u*, 1/sec; *q*, 0·1 sec. Electrical stimuli shown on lower lines of records *c–u*.

It may be noted that it proved impossible to make satisfactory observations on *Noctiluca* at sea when the ship was under way. The vibration of the vessel produced continuous excitation, and fatigued the luminescent response.

Fatigue

As others have observed before (e.g. Gosse, 1853), the flashing of *Noctiluca* is subject to fatigue. When one or a few Noctilucae are placed in a small dish and the dish is repeatedly shaken the cells flash several times, but after four or five flashes the light becomes weaker and disappears. Following a rest period of several minutes the cells recover and flash brightly once more.

Similar fatigue is shown under repetitive electrical stimulation, when the flashes decline in intensity, either initially from the first flash, or subsequent upon build-up to a plateau level (cf. histograms in Figs. 3 and 4). The time for recovery of full strength of response was determined by stimulating a cell at various intervals. The procedure was to give a pair of stimuli at some selected interval, and then to allow a rest period of 5–10 min before administering another pair of stimuli. The rest period of 5–10 min was chosen after some preliminary trials had shown that recovery occurred in that time.

With electrical stimulation it was found that recovery of full flash-intensity required an interval of about 2 min (19° C). Data obtained from one experiment in which a single cell was stimulated with pairs of shocks at given intervals are plotted in Fig. 5. Despite the scatter of observations, a general trend towards recovery of full luminescent intensity with lengthening of interval is apparent. The distribution of points representing flash-intensity at the 5 min level also gives some idea of the variation of response-intensity of a rested cell under electrical stimulation. It is uncertain whether this variability is intrinsic, or whether it is due to variation in apparent threshold resulting from small movements of the *Noctiluca* in the central well of the stimulating-chamber.

ELECTRICAL STIMULATION

A single electrical shock, above threshold-strength, elicits a flash of light from the cell or cells in the current-field (Fig. 2*c*). To bursts of shocks, a cell flashes repetitively, one flash per stimulus (Fig. 2*d–f*). With fast stimulation (>3/sec), some fusion of consecutive responses occurs, the effect becoming more pronounced as the frequency is raised (Fig. 2*e–g*). Fusion is nearly complete at a frequency of 28/sec (interval of 36 msec). This interval slightly exceeds the time required to reach maximal response-intensity, viz. 26 msec (latent period + rise time). Following bursts of high-frequency stimulation, there is usually a prolonged after-glow, lasting as long as $\frac{3}{4}$ sec (Fig. 2*g*).

Temporal characteristics

Single flashes, induced by electrical stimulation of resting cells, vary in duration from 69 to 830 msec. A flash lasting 145 msec has the following temporal characteristics: rise time, 17 msec; time to $\frac{1}{2}$ maximum, 8 msec; time of $\frac{1}{2}$ decay, 12 msec. Latent period, measured in records at maximal amplification, is 9 msec (temp. 19° C). Apart from latent period, these values agree reasonably well with those of responses evoked by mechanical

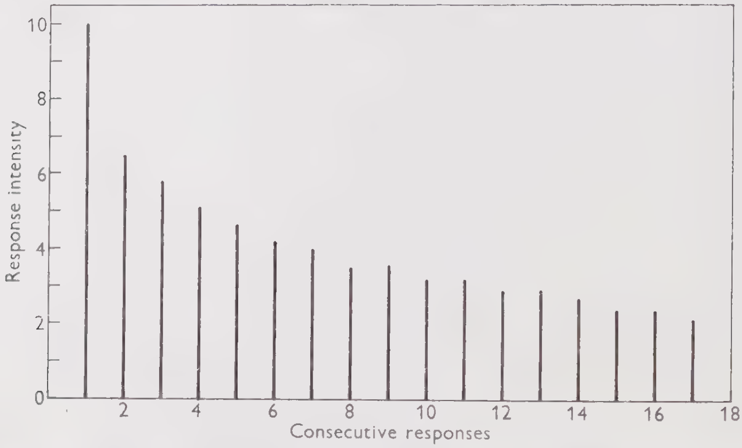

Fig. 3. Fatigue of photogeny in a Noctiluca cell. The cell was stimulated with a burst of 17 electric shocks at a frequency of 2/sec. Note progressive decline in height of responses. Ordinates, luminescent intensities in arbitrary units. Temp. 19° C.

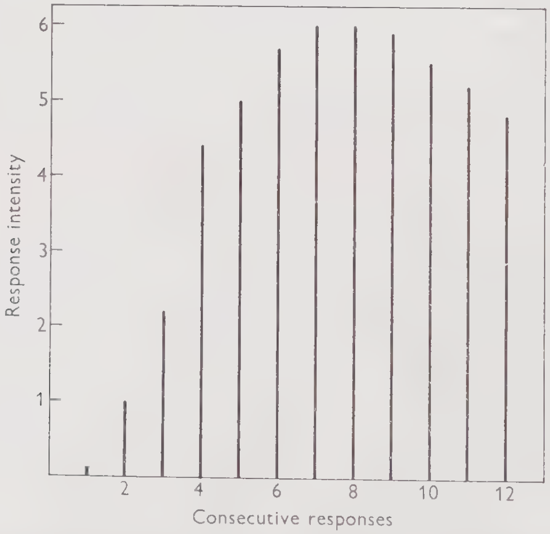

Fig. 4. Histogram showing facilitation and fatigue of consecutive responses in *Noctiluca*. A cell was stimulated with a series of 12 shocks at a frequency of 9/sec. Each vertical line represents the intensity of a flash measured from the level of luminescence (if any) existing at the beginning of that response. Intensities (ordinates) in arbitrary units. Temp. 20° C.

stimulation (p. 537). The latency after mechanical stimulation is discussed on p. 538.

The apparent duration of the flash as measured in the photographic records is influenced by the amplification of the apparatus. To determine the magnitude of this effect, flashes of a single cell were recorded at various amplifications and at fast paper speed. Since amplification of the apparatus varied in a linear manner, the responses at high amplification passed off the paper, and it was possible only to measure latent period and total duration of these responses. In order to secure a series of responses of approximately the same intensity, a rest period of 2 min was allowed between each flash, and conditions of stimulation were kept constant. The amplification factor was varied from 0·3 to 100.

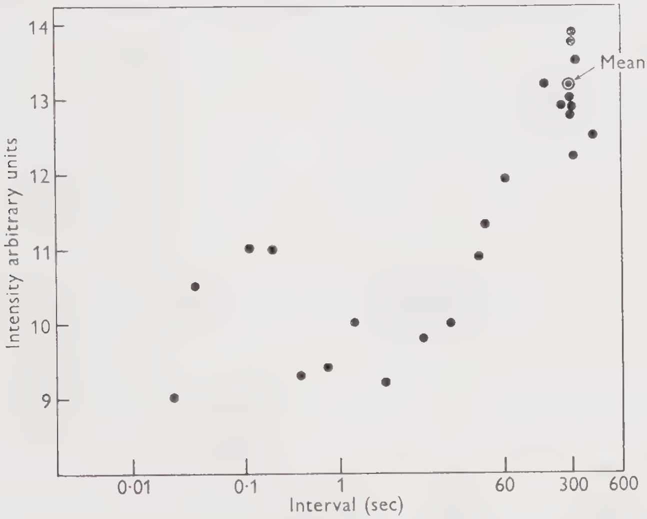

Fig. 5. Recovery of luminescence in a Noctiluca cell following electrical stimulation. Each point represents the intensity of a flash after a rest-period of selected duration since the last flash. Ordinates, intensities of response in arbitrary units. Abscissae, intervals in sec between 2 shocks (log $_{10}$ scale). Temp. 19·4° C.

The results are plotted in Fig. 6 A, in which it appears that the measurable duration of a flash is a high function of the amplification used. In the same figure there is a curve (B) showing apparent increase in duration of a brief flash from an artificial light source: actual exposures (flashes) had the same length (regulated by a mechanical device). When the amplification is increased by a factor of 100, the response-duration appears to be lengthened two-fold. The maximal response-duration in this series of records was 182 msec (19° C). The response-duration of 145 msec, mentioned on p. 539, was determined with an amplification-factor of 3. At maximal amplification of 100, this would appear 80 msec longer (i.e. 225 msec).

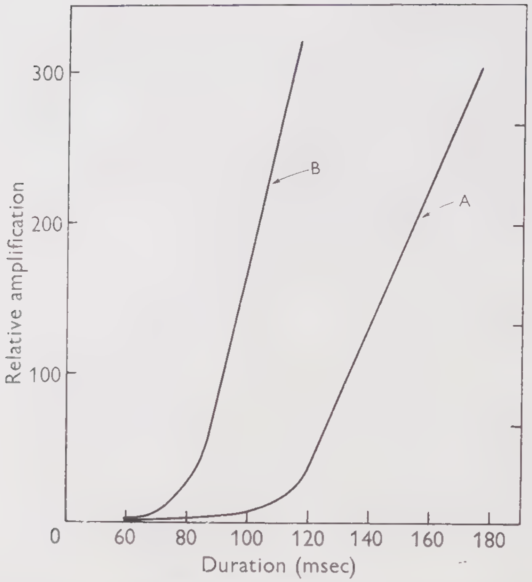

Fig. 6. Graph showing how the measurable duration of a flash appears to lengthen as the amplification is raised. Curve A, based on flashes of a single Noctiluca cell recorded at different amplifications (temp. 19° C). Curve B, based on exposures of equal duration of an artificial light source.

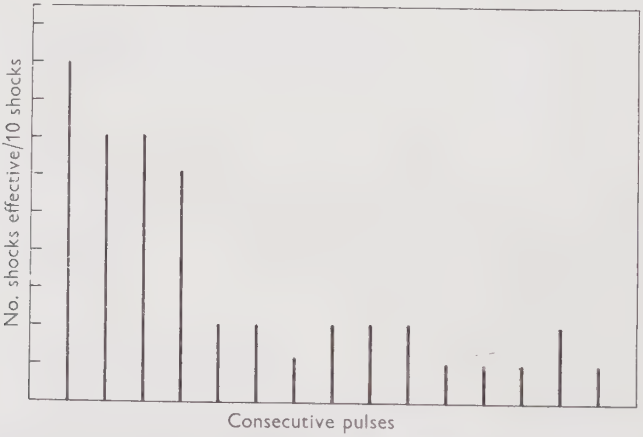

Fig. 7. Fluctuation of threshold. A Noctiluca cell was stimulated with a burst of 150 electrical pulses at a frequency of 3 sec. On the ordinates are plotted the number of stimuli effective in each consecutive series of 10 shocks (total scale = 10). With continued stimulation, fewer pulses were effective, until only each 4th or 5th shock produced a flash. Temp. 19° C.

Effect of increasing stimulus-strength

The intensity of a flash produced by *Noctiluca* depends on the strength of stimulus. This was determined either by varying the voltage, or by switching to different stimulating capacitors. When the current was gradually increased above threshold level, it was found that the response increased correspondingly (Fig. 2*h*–*n*). By doubling the stimulating voltage, the intensity of the luminescent flash was increased fivefold. A plateau-level of response was finally reached, above which further increment of stimulus-strength produced no further increase in flash-intensity.

At maximal stimulus-intensities the flashes were usually prolonged in duration as well as increased in intensity (Fig. 2*n*). Strong flashes, several times threshold-strength, sometimes gave rise to double flashes in quick succession (Fig. 2*o*). Continued stimulation at high voltages produced depression of the response, and finally extinction. The effect was irreversible, and indicative of cellular injury.

After a prolonged period of electrical stimulation it was usually found that the threshold was raised. This was also exhibited by giving a series of shocks at threshold-strength. During such a series occasional shocks became ineffective (Fig. 7). No evidence was found for summation of subthreshold stimuli: bursts of subthreshold shocks at various frequencies failed to elicit a response.

Facilitation

Noctiluca cells often but not invariably show facilitation under electrical stimulation. This is a phenomenon in which each stimulus engenders a persistent excitatory state which, when added to that produced by the next stimulus, augments the intensity of the next response. Under repetitive stimulation the successive flashes of a Noctiluca cell sometimes increase in intensity (Fig. 4).

Facilitatory increment occurred at intervals up to 1·4 sec, too long to allow fusion of separate responses, and increased at shorter intervals (Fig. 2*d*, *e*, *f*, *s*, *t*, *u*). During a long burst of stimuli, facilitation was evident initially for the first eight or so responses, whereupon the responses reached plateau level, which was briefly maintained and followed by a decline of response-intensity (Fig. 4). It has already been noted that the luminescent responses of *Noctiluca* are fatigued by repetitive stimulation at short intervals, recovery requiring some 2 min. This recovery period, during which the flash-intensity is depressed below normal, far exceeds the maximal interval at which facilitation is operative (viz. *ca.* 1·5 sec).

It is concluded, therefore, that facilitatory increment is partly masked by fatigue, i.e. the height of each facilitated response is the resultant of two conflicting factors, increment of excitation and temporary depression of the photogenic system.

In some other instances, facilitation was shown only in the second of a series of flashes, the third and subsequent flashes progressively decreasing in intensity (Fig. 2*q*, *r*). Again, there were many records in which the first flash of a series was maximal, and the subsequent flashes diminished in intensity (Figs. 2*p*, 3). This progressive diminution of successive flashes has been alluded to previously as fatigue (p. 539). I was unable to discover any apparent reason for this variability in the responses of different cells. The existence of facilitation in many cells, however, was well established, and is of intrinsic interest.

Illumination without effect

Exposure to light is said to cause inhibition of luminescence in *Noctiluca* (Henneguy, 1888) as well as certain other species (dinoflagellate *Gonyaulax* and ctenophores) (Harvey, 1952; Haxo & Sweeney, 1955). In view of the reported sensitivity of *Noctiluca* to light, it was decided to re-investigate the phenomenon. The procedure was as follows. A single Noctiluca cell or a group of cells was maintained in the dark for an hour, and then stimulated with an electrical shock to record the level of a normal response. The cell or cells were then exposed to a daylight tungsten bulb for 10–30 min at illumination levels up to *ca.* 1 kilolux. After extinguishing the light the Noctilucae were again stimulated electrically. The responses recorded were no weaker than those elicited before illumination. This result agrees with the observations of E. N. Harvey (1926) who found that daylight was without effect in suppressing the luminescence of *Noctiluca*. Massart (1893) reported a diurnal rhythm of luminescence in *Noctiluca* in continual darkness. I did not observe that the cells which I studied gave any brighter flashes by night than by day.

SPECTRAL COMPOSITION OF THE LIGHT

Dense layers of *Noctiluca* were obtained from plankton hauls, and the light produced by mechanical agitation of these samples was sufficiently bright and persistent for determination of its spectral composition. As an initial attempt, I examined the light of *Noctiluca* visually through a series of glass and gelatine filters (Chance, Ilford and Kodak), so as to judge the efficiency of the recording system. Filters used were purple (transmitting in the blue and red), green, yellow, orange, and red. The light of *Noctiluca* was largely eliminated by red and orange filters (transmitting above 590 mμ), faintly visible through blue and green filters (transmitting in the range 400–610 mμ). From these observations it appears that the light of *Noctiluca* has a spectral distribution between about 400–600 mμ. This range is in the spectral region to which the photomultiplier is most sensitive.

Relative spectral emission was determined quantitatively, as follows. The light from Noctilucae was passed through coloured spectral filters, and the resultant electrical responses were estimated by measuring oscilloscope deflexions. The spectral filters were mounted in openings about the margin of an opaque disc, which rotated beneath the photomultiplier. The speed of the disc was slightly faster than 1 rev./sec. Light was produced by discharging a suspension of Noctilucae from a pipette into a vessel lying below the disc. The experimental arrangement is depicted diagrammatically in another paper (Nicol, 1957*c*, fig. 1), where the method is described in more detail.

Two discs were used, each containing 8 or 9 different kinds of spectral filters. These are tabulated in another paper (Nicol, 1958*c*).

The filters were slightly wider than the diameter of the photomultiplier end-window. An opaque blank space between two contiguous filters allowed the responses of the individual filters to be distinguished; and a double blank at one place on the disc permitted a complete rotation to be determined, and the many responses during that rotation to be related to the corresponding filters on the disc. A sample record is shown in Fig. 8.

The flash produced by squirting a suspension of Noctilucae lasted about 3–4 sec, and during that time it changed greatly in intensity, rising quickly to a maximum, and then decaying. In order to follow changes in absolute intensity, identical filters, termed reference filters, were placed between each of the other spectral filters. The reference filters were Ilford blue-green 603 or Ilford green 604. The value for the measured response of each of the spectral filters was then altered, relative to the responses of the two contiguous reference filters.

Values for relative spectral energy were calculated from the photographic records. The method is described in detail in other papers (Nicol, 1957*b*, *c*, 1958*b*).

A curve for relative spectral emission is shown in Fig. 9. The light is blue: emission extends from about 420–580 mμ, with a maximum at 470 mμ. The curve is unimodal, and resembles that of luminous extracts of the dinoflagellate *Gonyaulax polyedra* (Hastings & Sweeney, 1957). Several other pelagic animals emit blue light of somewhat similar spectral composition (Kampa & Boden, 1957; Nicol, 1958*c*). It may be that a curve of this kind is fairly representative of the luminescence of many marine organisms. Ctenophores, with green light, are obvious exceptions.

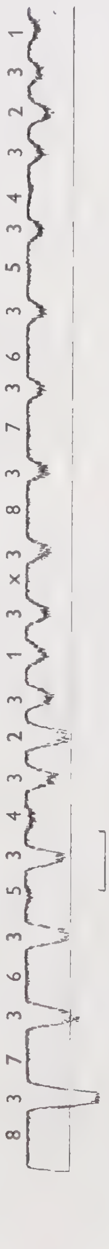

Fig. 8. Spectral analysis of the light of *Noctiluca*. Photographic strip showing deflexions corresponding to passage of spectral filters during a luminescent flash. Time below, 100 msec. Disc I, 1 revolution/sec. Ilford filters as follows: 1, 601; 2, 602; 3, 603; 4, 604; 5, 605; 6, 606; 7, 607; 8, 608; × double space as marker.

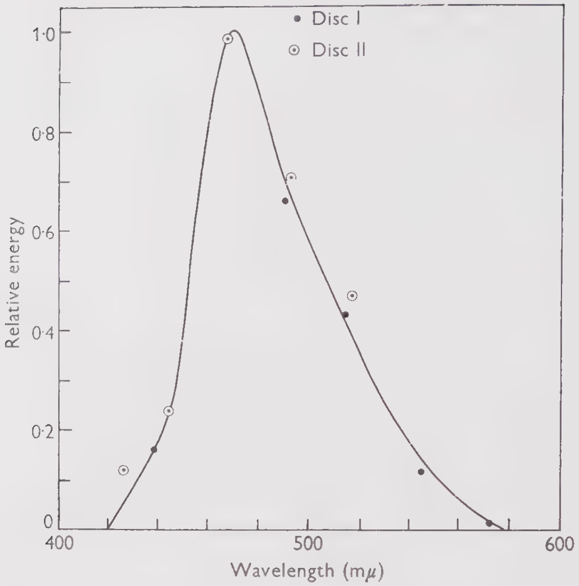

Fig. 9. Curve showing relative spectral composition of the light of *Noctiluca*.

INTENSITY OF FLASH

Estimations were made of the intensity of light in a flash of *Noctiluca*. These were computed from oscilloscope records of flashes of a single cell, excited electrically. The whole system was calibrated against a substandard light source. For daily use, a photometric standard consisting of a stilbene phosphor irradiated by ^{60}Co was used. Calibration and calculations are described elsewhere (Nicol, 1958*a*). Radiant flux in a single flash varied from $0{\cdot}03 \times 10^{-6}$ to $0{\cdot}16 \times 10^{-6}$ μJ/cm^2 receptor surface at 1 cm. Mean flux was $0{\cdot}1 \times 10^{-6}$ μJ/cm^2 receptor surface at 1 cm (23° C).

DISCUSSION

Observations on *Noctiluca* by E. B. Harvey (1917), and by myself in the present investigation, have revealed many points of similarity between the flashing of this unicellular organism, and of metazoans where luminescence is under nervous control.

The luminescence of *Noctiluca* is intracellular, and is evoked by external stimulation. The natural stimulus is mechanical, *Noctiluca* flashing when it is agitated or jarred. But flashing is also produced by electrical stimulation and there is likewise evidence that the photocytes of some higher animals are directly excitable by electrical pulses, e.g. those of polynoids when the nervous system is put out of action by anaesthesia (Nicol, 1957*a*). The luminescent systems of these animals can thus be added to the list of effectors which are directly excitable by electrical stimuli, viz. muscle, exocrine glands, flagella, and electrical tissue.

The flash of *Noctiluca* is very brief, usually about 100 msec following mechanical stimulation, but showing considerable variation. Some comparable data for the intracellular flashes of other animals, recorded from point sources of photogenic tissue, are assembled in Table 1. Of equal brevity are the flashes of *Gonyaulax* and of single scales of polynoids; in contrast is the prolonged glow of *Pyrosoma*, 7 sec or more in duration. The brief latency (about 10 msec) is comparable to that found in luminescent flashes of some metazoans, e.g. untreated and anaesthetized scales of polynoids, where the response is normally under nervous control.

TABLE 1. DURATION OF INTRACELLULAR FLASHES OF SOME MARINE ANIMALS

(Recordings from point sources.)

Animal	Duration	Temp. ° C	Reference
Gonyaulax polyedra	130 msec	—	Hastings & Sweeney, 1957
Noctiluca miliaris	22–95 msec	17	Original
Aequorea forskalea	0·2–0·4 sec	14–16	Davenport & Nicol, 1955
Renilla köllikeri	0·25–1 sec	17–20	Nicol, 1955
Beroë ovata	0·16 sec	20–21	Nicol, 1958*c*
Mnemiopsis leidyi	0·290 sec	21–23·5	Chang, 1954
Acholoë astericola	63–103 msec	18–20	Nicol, 1953
Pyrosoma atlanticum	7 sec	25	Nicol, 1958*c*

Under repetitive electrical stimulation, the flashes of *Noctiluca* show summation, fusion and facilitation, as do those of various higher forms, e.g. hydromedusae, sea pens, ctenophores and polynoids (Nicol, 1953; Chang, 1954; Davenport & Nicol, 1955). There has been some question whether the facilitatory process takes place within the photogenic cell, or in the nervous system, or whether it operates at the neurophotocyte boundary. The observations on *Noctiluca* show that facilitation can occur within the confines of a single photogenic cell. It has also been demonstrated to occur in anaesthetized elytra of polynoids, when the nerve-fibres are non-operative; in this instance, also, one must postulate a facilitatory process within the photocyte (Nicol, 1957*a*). Still to be resolved is the intracellular level at which facilitation operates, whether it involves the initial stages of excitation, or some later stages of the biochemical reactions producing the light.

The emission spectrum of Noctiluca light extends from about 420 to 580 mμ, with a peak at about 470 mμ. Clear oceanic water is most transparent to blue light of this composition, and it is in this region of the spectrum that the eyes of some deep-sea species are most sensitive, viz. squid and teleosts ($\lambda_{\text{max.}}$ 493 and 480 mμ, respectively) (Denton & Warren, 1957; Hubbard & St George, 1958).

The radiant flux in a flash of a *Noctiluca* ranges from $0{\cdot}03 \times 10^{-6}$ to

$0{\cdot}16 \times 10^{-6}$ μJ/cm² receptor surface at 1 cm. On the assumption that the cell emits uniformly in all directions, the total energy irradiated from a *Noctiluca* is $0{\cdot}38 \times 10^{-6}$ to $2{\cdot}01 \times 10^{-6}$ μJ/4π sterads. Values for radiant flux are available for two other protozoans, viz. the radiolarians *Cytocladus* and *Aulosphaera* which emit $0{\cdot}6 \times 10^{-5}$ to $5{\cdot}3 \times 10^{-5}$ μW/cm² receptor surface at 1 cm (Nicol, 1958*c*). Nichols (1924) has estimated the brightness of individual flashes of dinoflagellates at 0·116 mlam. When allowance is made for the blue composition of dinoflagellate light, this brightness estimate is equivalent, approximately, to a radiant flux of $0{\cdot}354 \times 10^{-5}$ μW/cm² receptor surface at 1 cm distance. Since the individual flashes of dinoflagellates are only some 0·1 sec in duration, equivalent flux in Nichols's estimates, averaged over 1 sec, is of the order of $0{\cdot}15 \times 10^{-6}$ μJ/cm² receptor surface at 1 cm distance. This shows reasonably good agreement with the present estimates of radiant flux of *Noctiluca*.

I am indebted to Dr M. Parke for undertaking the culture of *Noctiluca*, and to Mr E. I. Butler for technical assistance. Mr G. N. Harding of the U.K. Atomic Energy Authority supplied the ^{60}Co-stilbene phosphor, for which I am grateful. Part of the apparatus used in this research was purchased with grants from the Royal Society.

SUMMARY

The luminescent flashes of *Noctiluca miliaris* have been recorded by means of a blue-sensitive photomultiplier. Following mechanical stimulation, a cell gives a brief flash, lasting up to 90 msec. Repeated stimulation quickly leads to fatigue of photogeny; rest allows recovery. With electrical stimulation there is a flash for each pulse. With repetitive electrical stimulation, there is summation, then fusion of flashes, depending on the frequency. Some cells gave clear evidence of facilitation. Increasing the current produced brighter flashes. Illumination was without effect on the photogenic response.

The relative spectral composition of the light was determined by means of glass and gelatine filters. Emission extends from about 420 to 580 mμ, with a maximum at about 470 mμ. Radiant flux in a flash from a single cell ranges from $0{\cdot}03 \times 10^{-6}$ to $0{\cdot}16 \times 10^{-6}$ μJ/cm² receptor surface at 1 cm distance (23° C).

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OBSERVATIONS ON THE LUMINESCENCE OF *PENNATULA PHOSPHOREA*, WITH A NOTE ON THE LUMINESCENCE OF *VIRGULARIA MIRABILIS*

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(Text-figs. 1–8)

Pennatula phosphorea Linn. is a well-known luminescent animal, which was studied by that pioneer investigator, Panceri (1871, 1872*a*, *b*). He found that the animal luminesces when excited, and that the light emanates from the zooids. From the region stimulated luminous waves proceed, with measurable velocity, to the extremities of the animal. Since waves can be initiated in either direction, up or down the colony, it follows that the transmission system is non-polarized. Recent studies on other species of pennatulids have shown that luminescence is under control of a nerve net (Nicol, 1955*a*, *b*, *c*; Davenport & Nicol, 1956). Harvey's book on *Bioluminescence* (1952) gives a review of pertinent literature, plus a bibliography.

In the present study, the luminescent responses of *P. phosphorea* have been analysed by means of photoelectric recording, with special attention to the flashes of individual zooids. Spectral emission has been determined, and estimates made of the intensity of the luminescence of *P. phosphorea* and *Virgularia mirabilis*.

MATERIAL AND METHODS

Specimens of *Pennatula phosphorea* Linn. and *Virgularia mirabilis* O. F. Müll. were obtained in the otter trawl 15 miles south of Penmarch light, position 47° 32′–35′ N., 4° 11′–12′ W., depth 90 m. These were examined in the ship's laboratory at sea (R. V. 'Sarsia') or brought back to the Plymouth Laboratory.

Luminescence was recorded with photomultiplier tubes, E.M.I. types nos. 6685 and 6095B, connected to double-beam C.R.O.'s containing DC amplifiers. Oscilloscope deflexions were photographed on moving paper or stationary film. Spectral emission and radiant fluxes were determined with photomultiplier type no. 6685, the spectral sensitivity of which is given elsewhere (Nicol 1958*a*, fig. 2A). For the former measurements spectral filters were used, incorporated in a disc (no. II) and described in table 1 of another paper (Nicol 1958*c*). The methods of calculation are described in earlier papers (Nicol 1958*a*, *b*).

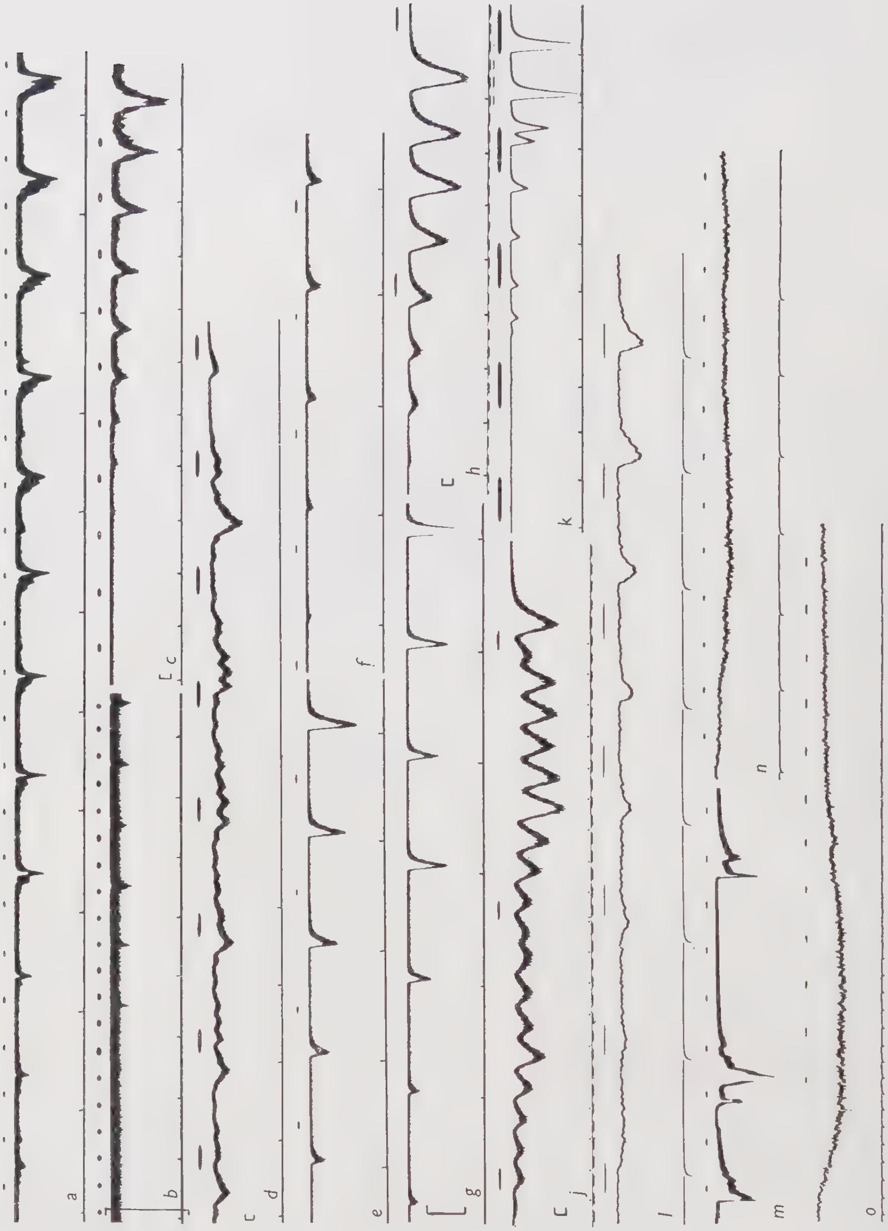

Fig. 1

Electrical stimuli were condenser shocks or square wave pulses, applied through Ag or Pt electrodes.

The observations which follow pertain to the autozooids of *Pennatula phosphorea*, unless indicated to the contrary.

Temperatures at which observations were made ranged from 18° to 20·8° C.

OBSERVATIONS

Both *Pennatula* and *Virgularia* are elongated sea pens, having a feather-like shape. There is a basal stalk or peduncle, from which arises the distal rachis. The latter has a central shaft, forming a support for a series of leaves on either side. The leaves of the rachis bear numerous autozooids, whereas the back of the shaft is covered with siphonozooids. *Virgularia* is long and whip-like compared with *Pennatula*.

When disturbed, *Pennatula* contracts; when left quiet, it takes in water and gradually swells. Observations were made on rested specimens, that had not been disturbed for an hour or more.

Light is emitted by the zooids (both autozooids and siphonozooids) and when the animal is excited, luminescent waves run over the length of the rachis. With persistent and repeated stimulation, the whole rachidial surface becomes aglow with a scintillating and shifting pattern of luminous points. The response is quickly fatigued, and time is required for recovery.

ELECTRICAL STIMULATION

Under repetitive electrical stimulation *Pennatula* responds with luminous waves which run over the surface of the colony from the region of the electrodes (Fig. 1*a*). Usually a response appears after the first shock, sometimes after the second or third shock. At slow frequencies (intervals > 1 sec), there

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Legend to Fig. 1

Fig. 1. Oscilloscope recordings of the luminous flashes of sea pens. Time scale above each record, 1/sec; downward deflexion of middle trace represents luminous response; pips on lower line, if any, are electrical stimuli. Photographs (positives) made on moving paper. (*a*) *Pennatula phosphorea*. Recording of transmitted waves from autozooids of whole animal. Burst of 12 shocks at 1/2 sec. Temp. 18·5° C. (*b*, *c*, *d*) *Pennatula*. Responses of siphonozooids in the intact animal to various rates of stimulation (1/2 sec, 1/sec, 90/min, respectively). *d* is latter part of a longer record, beginning with the 13th pulse, and shows much flickering and after-discharge. Relative amplification given by height of vertical lines. Temp. 20° C. (*e*, *f*) Flashes of an isolated autozooid of *Pennatula*, showing fatigue. The autozooid was stimulated with burst of 10 shocks (1/sec), 2 min interval between bursts. *e*, 2nd burst; *f*, 7th burst. Last 5 shocks only shown. Temp. 20·8° C. (*g*, *h*, *j*) Responses of an isolated autozooid of *Pennatula* to electrical stimulation at various frequencies. *g*, burst at 1/sec, last 7 pulses of 14; *h*, 4/sec, last 8 pulses of 12; *j*, 9/sec, last 20 pulses of 24. Relative amplification shown by vertical lines. Temp. 20·8° C. (*k*) Isolated autozooid of *Pennatula*. Burst of pulses at 132/min. Note double flashes. Temp. 20° C. (*l*) Localized responses of siphonozooids of *Pennatula*. Burst of shocks at 72/min. Temp. 20° C. (*m*) *Virgularia mirabilis*. Transmitted waves elicited by mechanical stimulation. Temp. 17° C. (*n*, *o*) *Virgularia*. Bursts of pulses at 36/min and 3/sec. Low band pass filter. Recording from a stretch about 5 cm long. Temp. 19° C.

is 1 flash/shock, and successive flashes increase progressively in intensity (Fig. 1*a*). The response pattern is often rather irregular when stimulus intervals are long (Fig. 2).

At slow frequencies, each response has time to return to base-line, thus excluding summation, or the build-up of flash intensity by one response being superposed on its predecessor. Despite the absence of summation, there is still an increment of flash intensity in consecutive responses, an increment which can be ascribed to facilitation (Fig. 1*a*) (Pantin, 1952). The same patterns of responses are shown by other sea pens, e.g. *Renilla* and *Leioptilus* (Nicol, 1955*a*, *b*; Davenport & Nicol, 1956).

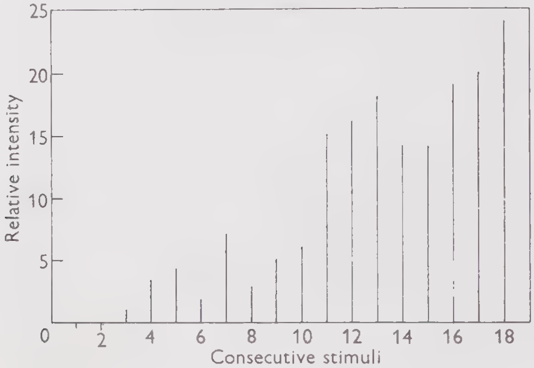

Fig. 2. Histogram showing increase in height of successive flashes of autozooids (*Pennatula*). Recording from whole animal stimulated with a burst of shocks at 48/min. Temp. 18·2° C.

Facilitation lasts at least 1 min. An animal was stimulated with bursts of shocks (frequency 1-2/sec), the several bursts being separated by intervals of 30 sec or 1 min. The responses in the second series were brighter than those in the first, and those in the third series brighter still. The enhancing or facilitatory effect is carried over from one period to the next, despite the long intervening intervals of quiescence.

After a burst of impulses, there is often a certain amount of after-discharge, in which several luminous waves continue to arise after stimulation has ceased (cf. Fig. 1*d*). The luminous waves in a bout of post-stimulatory flashing gradually decrease in frequency and amplitude.

The latency after electrical stimulation was measured as follows. An animal was covered, except for a slit 1 cm wide across the body, and the electrodes were placed at this level. The animal was stimulated with a burst of shocks at 1/sec, and responses were recorded at maximal amplification. The mean latent period, in 8 responses, was 0·25 sec; variations in measurements were ± 0·02 sec, and were random.

When recordings were made from a slit lying some distance from the electrodes, it was found that the latency of the flash increased markedly during a long burst of pulses. Some actual examples are as follows:—

Burst of 15 shocks at 40/min. Latency increased from 0·76 to 1·54 sec, i.e. an increment of 2 times.

Burst of 18 shocks at 4/sec. Latency increased from 1·2 to 3·2 sec, i.e. an increment of 2·7 times

These recordings were made from a 1 cm aperture on the rachis. Since the electrodes were some distance away, the latencies include conduction time to the region from which the flashes were recorded.

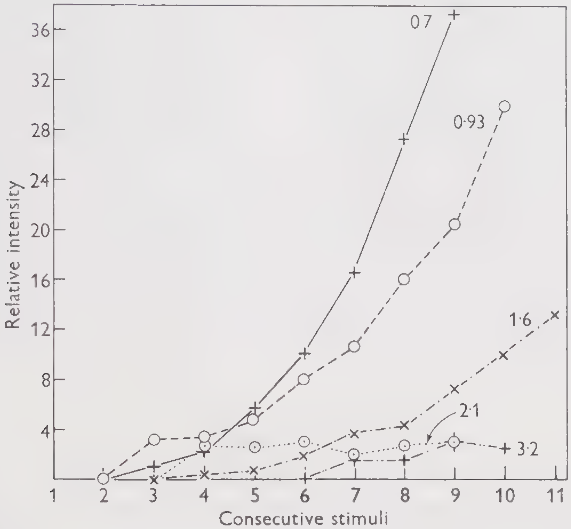

Fig. 3. Facilitation in siphonozooids of *Pennatula*. Curves showing how the amplitude of consecutive responses increases, and the effect of different frequencies of stimulation. Figures on the curves give the interval between stimuli. Recording of responses of siphonozooids of an intact animal. Temp. 20° C.

Responses of siphonozooids

Luminescent responses of the siphonozooids are similar to those of autozooids in the intact animal. Repetitive stimulation evokes luminescent waves that are propagated along the back of the rachidial stem. These increase progressively in intensity, and show the same pattern of facilitation as do the autozooids (Fig. 1 *b–d*). The rate of facilitatory increment becomes greater as the frequency is raised (Fig. 3). Following prolonged stimulation there is much after-discharge (Fig. 1 *d*).

Temporal characteristics of flash

Recordings were made from a slit, 8 mm wide, extending across a sea pen (*Pennatula*). The animal was stimulated electrically. Total duration of response was 0·7 sec; rise to maximum occupied 0·2 sec; time to ½ maximum was 0·14 sec; decay from maximum was 0·5 sec. (18·4 °C). Even in this small field, of course, many autozooids were contributing to the flash.

Conduction velocity

I made some measurements of conduction velocity, by recording from two slits at a known distance. One or two photomultipliers were used, the latter arrangement being more satisfactory. Average conduction speed was 6·4 ± 1·4 cm/sec (18°–20° C). A visual estimation of conduction speed is probably at least as accurate, owing to difficulties in interpreting the records obtained by electronic means. Previous estimates gave a velocity of 5 cm/sec for the luminous waves of *P. phosphorea* (Panceri, 1871, temp. ?; Moore, 1926, temp. 15° C).

Illumination without effect

Exposure to light does not affect the luminescent responses of *Pennatula*. Three specimens were left under artificial light (tungsten bulb) for ½ h. Under mechanical stimulation waves of light were produced which ran over the animal and, with continued stimulation, these waves were succeeded by flickering light. Also, when animals are brought into a dark room from daylight, and then stimulated mechanically, they show normal, transmitted luminescent waves.

Local responses

When pieces, containing zooids, are isolated from the animal and stimulated electrically, the luminous responses remain localized in the vicinity of the electrodes. In such pieces, luminous flashes were never transmitted, either from autozooid to autozooid, or along a strip of siphonozooids. Preparations which behaved in this way were isolated under $MgCl_2$-anaesthesia, washed in sea water, and allowed long periods for recovery. It would seem that transmission in that part of the nervous system concerned with the luminous response becomes disorganized in an isolated piece of tissue.

Autozooids

When a pinna is cut off and electrodes are placed on one of the autozooids, only the autozooid immediately under the electrodes responds. No transmission of luminescence is visible in such isolated pieces. The following observations were made on excised autozooids of *Pennatula*.

With repetitive electrical stimulation an autozooid flashes each time it receives a shock, and consecutive flashes increase progressively in intensity (Figs. 1*e*, 4, 5). This is facilitatory increment. Occasionally an autozooid flashes twice in response to one shock (Fig. 1*k*). At fast rates of stimulation, the responses run into each other, and fusion is nearly complete at a frequency of 10/sec. Continued stimulation leads to fatigue, and the intensities of the flashes decrease. This effect is well seen when an autozooid is stimulated at regular intervals with identical bursts of shocks. In the later series the flashes gradually diminish (Fig. 1 *e*, *f*).

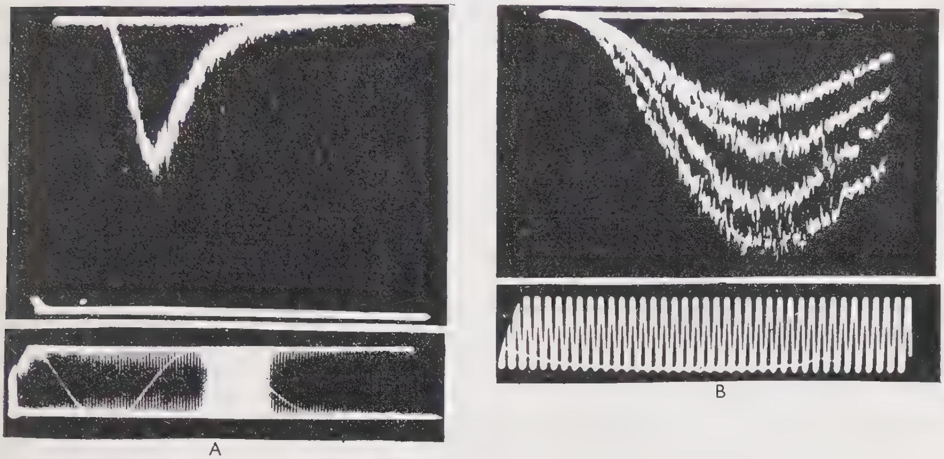

Fig. 4. Responses of an isolated autozooid of *Pennatula* to a single shock (A), and a burst at 2/sec (B). Sweeps. Time scale, 50 ~. Note facilitation in B. Temp. 20° C.

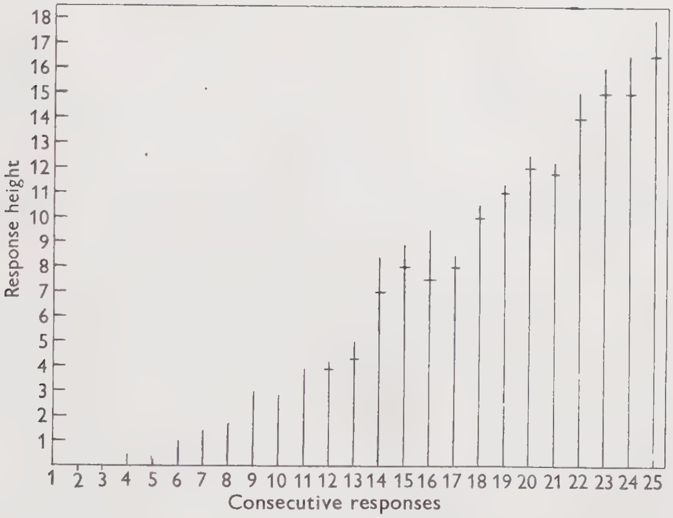

Fig. 5. Histogram showing changes in flash intensity of an isolated autozooid of *Pennatula* under repetitive electrical stimulation. Bursts of 25 shocks at 39/min. The cross-marks on the vertical lines represent response-height less residual luminescence obtaining at the start of the response. Temp. 20° C.

Facilitatory increment becomes more pronounced as the frequency is raised (Figs. 1*h–k*, 6). At very short intervals (around 0·1 sec) the process is reversed, the responses become irregular, and amplitudes are smaller than at longer intervals. The following measurements, as an illustration, refer to the responses of an autozooid stimulated with bursts of electrical shocks. The amplitude in each case is that of the response to the 10th shock in a burst:

Interval between stimuli	Amplitude (relative)
0·106 sec	14·27
0·200	42·7
0·371	20
0·970	5

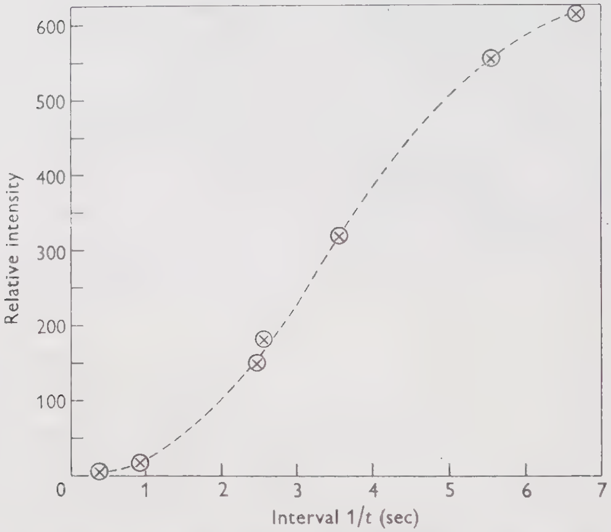

Fig. 6. Relation of flash-intensity to frequency of stimulation. Data derived from flashes of a single autozooid. Each point represents the intensity of the response to the 9th shock in a series. Abscissae, interval between shocks (1/*t*). Temp. 20° C.

The intensity of the flash varies with the voltage used, up to some maximum. In the present experiment this was 50 V. When the voltage was raised from 20 V to 50 V, the intensity of the flash increased 3 times.

The flash of an autozooid is brief, about 0·23 sec. Latency lies between 20 and 30 msec and is constant. The response rises to a maximum in about 39 msec; $\frac{1}{2}$ maximum is 15 sec; $\frac{1}{2}$ decay time from maximum is 63 sec.

Attempts were made to anaesthetize the autozooids, in order to find out more about the avenues of excitation, with little success.

Autozooids were placed in the following drugs for 3–4 h: cocaine, 0·1 %; procaine hydrochloride, 0·5 %; MS 222 (Sandoz), 0·1 %; novocaine, 0·5 %; chloretone, 0·1 %; isosmotic $MgCl_2$: sea water, 1·1. There were very weak responses in autozooids poisoned with cocaine and chloretone, none in the others. To a burst of shocks, the autozooid in cocaine gave a series of flashes, 1/stimulus; the first was brightest, and succeeding ones fell off in amplitude. Responses in chloretone were too weak to resolve satisfactorily. All these materials depress excitability too much to yield much information about the luminescent responses.

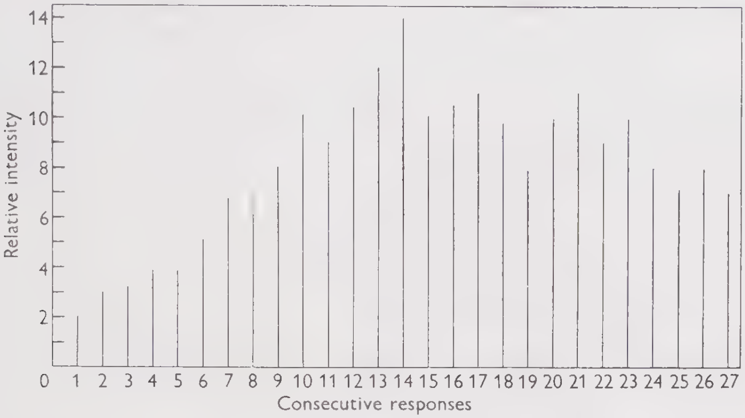

Fig. 7. Change in amplitude of consecutive flashes of siphonozooids (*Pennatula*). Localized responses from an isolated preparation of siphonozooids. Bursts of 27 shocks at 72/min. Ordinates, relative intensity; abscissae, consecutive responses. Temp. 20° C.

Siphonozooids

Isolated preparations of siphonozooids were made by cutting strips from the back of the rachidial stem. Under electrical stimulation luminescent responses are localized to the immediate vicinity of the electrodes. Each shock brings out a flash, the first response is relatively bright and prolonged, slowly reaching maximum; later flashes tend to be briefer, and to increase progressively in amplitude (Fig. 7). The increase in flash intensity occurs at slow rates of stimulation, and suggests that facilitation is operative in these instances (Fig. 1 *l*). In a prolonged burst fatigue sets in, and later flashes decrease in amplitude.

Latency, in localized responses of siphonozooids, is about 50 msec. In the first flash of a series, total duration was > 1 sec; time to maximum was 0·22 sec. The 14th flash of a series had a duration of 0·43 sec; rise to maximum occupied 0·078 sec (20 °C). There was no obvious increase in latency of consecutive responses. When high rates of stimulation were used, individual flashes could still be resolved at intervals of 0·1 sec.

LUMINESCENT RESPONSES OF *VIRGULARIA MIRABILIS*

Earlier workers were of the opinion that *Virgularia* is non-phosphorescent (Hickson, 1916, p. 154). *V. mirabilis*, however, is luminescent, but the light is very transitory, and the animal's photogenic ability is easily fatigued. In these respects it resembles *Stylatula elongata*, belonging to the same family (Virgulariidae) (Davenport & Nicol, 1956).

When mechanically stimulated—by gentle squeezing—*Virgularia* emits a faint blue glow, which is transmitted a short distance along the zooids (Fig. 1 *m*). When the animal is stimulated several times in succession, the light becomes feebler and finally ceases. When stimulated electrically, the animal emits flashes, beginning with the first or second shock; successive responses tend to fuse together (Fig. 1 *n*, *o*). The luminescent ability of this species is much inferior to that of *Pennatula*, and its responses are difficult to analyse satisfactorily.

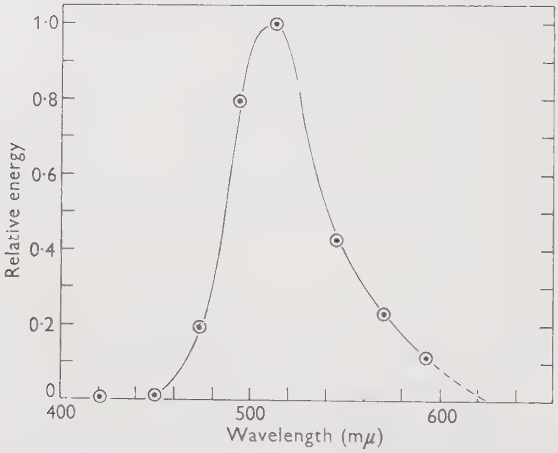

Fig. 8. Relative spectral emission of *Pennatula phosphorea*.

RELATIVE SPECTRAL EMISSION AND RADIANT FLUX

Spectral emission in *Pennatula phosphorea* extends from about 450 to 620 mμ. Maximal emission occurs at about 510 mμ (Fig. 8). The light is blue-green in colour.

Using the relative spectral emission curve shown in Fig. 8, estimates have been made of the intensity of light emitted by *Pennatula* and *Virgularia*. The light of *Virgularia* appears blue to the eye, and it is assumed that its spectral composition resembles that of *Pennatula*. Any difference is unlikely to be sufficient to introduce much error into the estimates of radiant flux.

Pennatula phosphorea. During electrical stimulation, the separate flashes increase progressively in intensity, owing to facilitation (p. 554). Flashes, recorded from the whole animal, last 0·4–0·7 sec (Fig. 1*a*). Radiant flux, in a series of 12 flashes, increased from $0{\cdot}84 \times 10^{-6}$ to $6{\cdot}96 \times 10^{-6}$ μJ/cm^2 receptor surface at 1 cm distance (18·5° C.)

Measurements were made of the intensity of light in flashes of isolated autozooids following electrical stimulation. Radiant flux in a single flash varied from $0{\cdot}096 \times 10^{-9}$ to $62{\cdot}80 \times 10^{-9}$ μJ/cm^2 receptor surface at 1 cm distance (temp. 20·8° C). The first value refers to the smallest measurable response in a series, the second to a maximal facilitated response.

Virgularia mirabilis. The luminescent response of *Virgularia*, evoked by mechanical stimulation, lasts about $1\frac{1}{2}$ sec. Estimated radiant flux, at 1 cm distance, is $1{\cdot}4 \times 10^{-4}$ to 12×10^{-4} μW/cm^2 receptor surface.

DISCUSSION

When *Pennatula* is stimulated waves of light appear which are propagated over the surface of the rachis. The luminescent response is followed by contraction of the animal and expulsion of water. The transmission system responsible for propagation of the luminescent response is presumably nervous; direct proof of this would be best afforded by electric recording. Propagation takes place in any direction (*Pennatula* and other sea pens), indicating that a non-polarized system is involved. This system could be a neural network possessing two-way synapses, i.e. interneural junctions capable of transmitting with equal facility in either direction. The luminescent response is propagated uniformly and without decrement; that part of the neural network involved, therefore, would appear to partake of the nature of a through-conduction system (cf. Pantin, 1950).

Horridge (1957) has investigated the retractile responses of alcyonarian, zoanthid and madreporarian polyps. In most groups there is extensive co-ordination over the colony, and in astraeid corals and *Tubipora* the polyps are linked by a through-conducting system. There appears to be little or no co-ordination of the retractile responses of the polyps of the sea pansy *Renilla*, according to Parker (1919). Peristaltic waves of contraction have been noted in this animal, but these are transmitted at much slower velocities than the wave of luminescence (Parker, 1920). Sea pens possibly possess several transmitting systems, each of which is concerned with regulating a particular kind of response, and to test this, or alternative ideas, an analytical study of the various responses of *Renilla* would be well worth while.

One of the most noteworthy features of the luminescence of sea pens is the progressive increase in the intensity of consecutive responses, as revealed by photoelectric recordings. This increment, termed facilitation, is revealed in localized recording from the intact animal, and in the responses of a single isolated zooid. Now, it is an interesting fact that when a piece of tissue, containing several zooids, is removed from *Pennatula* and is stimulated electrically, only the zooid immediately under the electrodes responds, and no transmission of excitation occurs. Therefore, one may argue, the nerve net is inexcitable in a severed fragment, and the photocytes are responding directly to electrical stimulation. When recordings are made of the flashes of isolated autozooids, it is found that these records show a pattern similar to those recorded from the intact animal. Latency is short, presumably because no transmission time is involved, and facilitation is evident.

In a previous paper dealing with luminescence in *Renilla* (Nicol, 1955*b*), evidence was presented that facilitation in that animal involved recruitment of photogenic loci with continued stimulation. Two obvious possibilities relating to facilitation in an isolated autozooid of *Pennatula* are: (*a*) individual photocytes have different levels of excitability, some of them responding to a single shock, others requiring many shocks before they respond; continued stimulation, therefore, leads to recruitment of photocytes, and greater light emission; (*b*) facilitation is an intracellular process involving a progressive increase of excitation in all the photocytes with continued stimulation. The increase in flash-intensity which accompanies a rise of voltage could be explained either way. Intracellular facilitation involving a single cell has been discovered in the flashes of *Noctiluca* (Nicol, 1958*d*), which provides an analogy for a similar phenomenon in *Pennatula*.

Another feature brought out by photo-electric recording is latency. In flashes from a single autozooid the latent period remains constant. But when records of transmitted waves are taken from the intact animal, measured latency from the stimulus to incidence of light emission shows progressive lengthening during a series of pulses. Since transmission through a series of neurones is involved in the latter preparation, but not in the former, it appears that the increase of latent period takes place in the nerve net. Possibly an increase in conduction time is involved, but the records are ambiguous in this respect.

The light of *Pennatula phosphorea* is blue green in colour. Other animals with similar spectral emission are ctenophores and polynoids (Nicol, 1957, 1958*c*). Values for radiant flux, ranging up to 7×10^{-6} μJ/cm^2 receptor surface at 1 cm, are of about the same magnitude as those for flashes of a single polynoid elytrum (Nicol 1958*a*).

SUMMARY

The luminescent responses of *Pennatula phosphorea* and *Virgularia mirabilis* were investigated by means of photo-electric recording. *Pennatula* produces luminescent waves which are transmitted over the surface at 6·4 cm/sec (20° C). Facilitation is shown in responses of the whole animal and in responses of isolated autozooids and siphonozooids. Data are given for latency and flash-duration. *Virgularia* is more easily fatigued than *Pennatula*. Spectral emis-

sion of *Pennatula* extends from about 450 to 620 mμ, with a peak at 510 mμ. Values for radiant flux are:

P phosphorea, $0{\cdot}8 \times 10^{-6}$ to 7×10^{-6} μJ/cm^2 receptor surface at 1 cm; *V. mirabilis*, $1{\cdot}4 \times 10^{-4}$ to 12×10^{-4} μW/cm^2 receptor surface at 1 cm (responses of the intact animal). A flash from a single autozooid of *Pennatula* has an intensity of $0{\cdot}1 \times 10^{\ 9}$ to $62{\cdot}8 \times 10^{-9}$ μJ/cm^2 receptor surface at 1 cm.

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NOTES ON STARFISH ON AN ESSEX OYSTER BED

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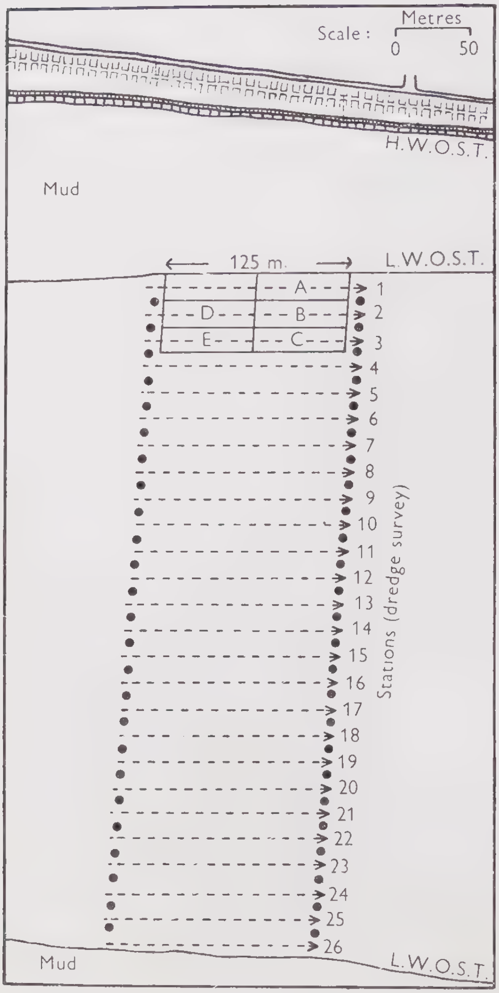
(Text-figs. 1–9)

In a previous paper (Hancock, 1955) an account was given of the feeding behaviour of the starfish *Asterias rubens* L. and the common sunstar *Solaster papposus* (L.) on Essex oyster beds. In discussion, it was stated that there was no evidence that, in the conditions described, a cultivated oyster ground provided a greater attraction than an uncultivated one. The present work was undertaken to provide information on this subject, and also on the movements, growth and ecological relationships of starfish. Further experiments were made on feeding behaviour, particularly of the young starfish.

Samples required to give information on the growth and distribution of starfish were obtained from regular surveys of an oyster bed, by a series of parallel dredge hauls covering both cultivated and derelict bottoms. In November 1954, a section of oyster ground, 125 m wide, and stretching from one bank to the other (Fig. 1), was marked out at the Southward Laying, River Crouch. The first dredge haul was made with two 4 ft. 6 in. dredges over the 125 m width, parallel to the edge of the north shore at L.W.O.S.T. and 10 m from it. Buoys were used to mark distances offshore, and subsequent dredge hauls were made parallel with each other 20 m apart, and, when time permitted, were continued as far as the south shore, giving a total of twenty-six stations. This procedure was repeated approximately monthly. The survey dates were chosen with tides of about the same height.

DESCRIPTION OF THE BOTTOM

At the Southward Laying, an area below L.W.O.S.T. is fully cultivated; most of the slipper limpets (*Crepidula*) have been removed and oysters have been relaid. Farther offshore the ground becomes semi-derelict with more *Crepidula*, and towards midstream is covered by an almost pure population of *Crepidula* (Table 1, Stations 6–11). In mid-river there is a submerged bank shallowest at Station 16 (Fig. 2) where, although little cultivation is done, tidal scour has made the bottom less muddy, and there is plenty of shell with large quantities of 'ross' or *Sabellaria*. There are some oysters, and, although *Crepidula* is present, the bottom is very much cleaner than in the deeper water over stations 6–11. The nature of the bottom at each dredge station is given in Table 1.


Scale :
Metres
0
50
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Mud
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A
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21
22
23
24
25
26
Stations (dredge survey)
Mud
L.W.O.S.T.

Results of grab survey
(Showing numbers of *Asterias* in
approx. position of grab hauls)

I. Stations A E: 10 grabs per station

						2	2	4	12	0	A
						4	1	11	0	0	
D	2	2	3	2	2	1	1	3	1	3	B
	3	0	2	1	1	4	3	3	5	3	
E	2	9	1	8	3	6	7	4	9	15	C
	0	5	4	5	6	7	8	1	0	2	

II. Stations D B (repeat): 5 grabs per station

D	5	6	3	6	2	6	11	22	1	3	B

Fig. 1. Map showing part of the Southward Laying, River Crouch used for dredge surveys, recapture experiment and grab sampling. Dotted lines show positions of parallel dredge hauls 1–26 between buoys (●). Enclosed rectangles (A–E) show areas surveyed by grab sampler. Areas B and D (dredge Station 2) were also used for the recapture experiment (see text). The results of the grab survey are also shown diagrammatically.

DISTRIBUTION OF ADULT STARFISH

From the results of the surveys the distribution of *Asterias* and *Solaster* was plotted (Fig. 2). Figures for bottom animals taken in dredges should be compared with caution because a dredge is not a fully reliable sampler (Shelbourne, 1957). However, the regular appearance of concentrations of particular animals on certain sections of the river suggests that in this instance, the dredge gave a satisfactory distribution picture.

It was shown previously (Hancock, 1955) that during 1954 the largest numbers of adult *Asterias* were taken from stations 3–6 (50-110 m below L.W.O.S.T., Fig. 2). Later, during 1955 and in 1956, the main area of abundance shifted offshore, perhaps as a result of the removal by dredging of large numbers of *Crepidula*, close to the cultivated ground, causing the *Asterias* to seek more abundant food farther offshore. No evidence was obtained of extensive migrations, and, particularly, there was no mass movement of adult *Asterias* to the cultivated oyster ground inshore just below L.W.O.S.T. The population was maintained just offshore in association with large numbers of *Crepidula*.

TABLE 1. SOUTHWARD LAYING, RIVER CROUCH. NATURE OF BOTTOM SHOWN BY DREDGING ON STATIONS 1–26.

	Nature of bottom	
Station	1955	1956
1	Shell, oysters, few *Crepidula*	Shell, oysters, very few *Crepidula*
2	Shell, oysters, few *Crepidula*	Shell, oysters, very few *Crepidula*
3	Shell, oysters, *Crepidula*	Shell, oysters, very few *Crepidula*
4	Shell, *Crepidula*, some oysters	Shell, oysters, few *Crepidula*
5	Shell, *Crepidula*	Shell, *Crepidula*, oysters
6	Shell, many *Crepidula*, muddy	Shell, *Crepidula*, oysters
7	Shell, many *Crepidula*, muddy	Shell, *Crepidula*, muddy
8	Shell, many *Crepidula*, muddy	Shell, *Crepidula*, muddy
9	Shell, *Crepidula*	As 1955
10	Shell, *Crepidula*	As 1955
11	Shell, *Crepidula*	As 1955
12	Shell, *Sabellaria*, *Crepidula*, some oysters	As 1955
13	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
14	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
15	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
16	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
17	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
18	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
19	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
20	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
21	Shell, *Sabellaria*, few *Crepidula* and oysters	As 1955
22	Clean shell, *Sabellaria*, few oysters	As 1955
23	Shell, *Sabellaria*, some stones	As 1955
24	Shell, *Sabellaria*, stones and mud	As 1955
25	Shell, *Sabellaria*, stones and mud	As 1955
26	Hard mud and boulders, some shell	As 1955

Solaster, however, had quite a different distribution and there was little overlapping between the adult populations of *Asterias* and *Solaster* (Fig. 2). The largest numbers of adult *Solaster* occurred throughout the year on grounds commencing 180 m from the north shore (Station 10 onwards) and extending to the opposite shore. They were not associated with the cultivated oyster ground near the north shore, nor with the main *Crepidula* zone (Stations 6–11). *Solaster* was mainly found on the submerged bank (Fig. 1), where

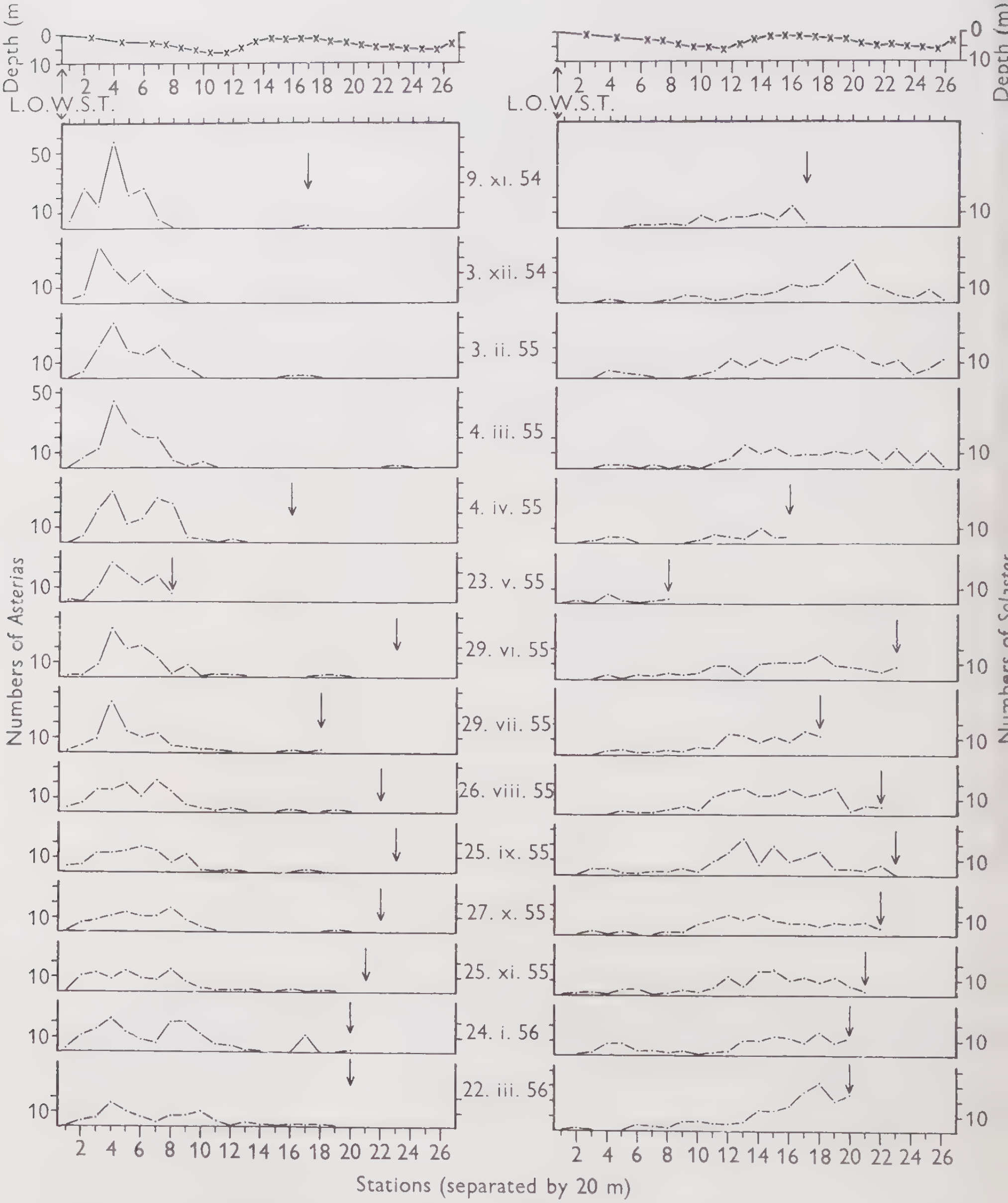

Fig. 2. Numbers of adult *Asterias* and *Solaster* taken using two 4 ft. 6 in. power dredges in parallel dredge hauls across the River Crouch at the Southward Laying 1954–56, commencing 10 m from L.W.O.S.T. on the north shore. Sections across the river showing mean depths are given. The final dredge station on each date is indicated by an arrow.

the bottom was of 'ross' (*Sabellaria*), some *Crepidula* and oysters, with *Alcyonidium*, *Sertularia*, *Dendrodoa* and some barnacles. The type of bottom at each station is shown in Table 1.

DISTRIBUTION AND SETTING OF YOUNG STARFISH

Juvenile *Asterias* were taken in 1955, for the first time, on 29 July, when they were 1–7 mm in maximum radius (Figs. 3, 5 and 6). By 'maximum radius' is meant the length of the largest arm from its tip to the centre of the mouth. The distribution of juveniles on the stations at the Southward Laying was compared at first by counting the population on fifty oyster shells taken at random from the dredge hauls on each station. On 29 July the largest numbers were taken on stations 2 and 3, with little settlement offshore (Fig. 3). Further settlement had occurred by 26 August when numbers had increased on all stations, but particularly offshore. When sampled on 25 November the juveniles had grown so much (Fig. 5) that they became easily detached from shells in the dredges, and the numbers on shells could no longer be used for comparison. On this and subsequent dates, the density of young starfish was estimated by subsampling the dredge contents, and calculating the numbers in two full dredges. These numbers could be compared more easily with the numbers of adult starfish (Fig. 2). Most juveniles continued to be found on inshore stations, with a fairly large settlement on Stations 12–20.

Juvenile *Solaster* appeared earlier in the year than *Asterias*, on 23 May 1955. This was expected, as in the laboratory *Solaster* spawned from 25 to 27 February 1954 at a mean temperature of 9° C, and on 16 March 1955 when the temperature was again 9° C. *Asterias*, however, did not spawn in 1954 until 29 May, when the temperature had reached 15° C. *Solaster* eggs spawned on 25 February 1954, kept under observation in the laboratory, were floating orange spheres 0·9 mm in diameter. By 2 March they had become gastrulae, which elongated in a day or two, and by 10 March had developed three larval arms (as figured by Gemmill, 1912, for *Solaster endeca*, and by Chadwick, 1914). Metamorphosed stages, less than 1 mm in diameter, with the first tube feet, were found attached to the bottom of the tank early in April. *Solaster* eggs were taken in the plankton from the River Crouch in 1956 for the first time on 26 March. The distribution of young *Solaster* at the Southward Laying was very similar to that of the adults, the greatest settlement being consistently about 250 m from the north shore (Fig. 3, Stations 11–14).

The numbers of *Asterias* settling in 1955 were much greater than those of *Solaster*. The greatest quantity taken during the year in two full dredges was 2724 on Station 2 on 23 September. The corresponding total for *Solaster* was 42 taken on Station 11 on 27 October 1955. In 1956, no settlement of *Asterias* was observed. Small numbers of *Solaster* settled, but they were not seen until 4 June, when they were considerably smaller than the previous year group was in June 1955.

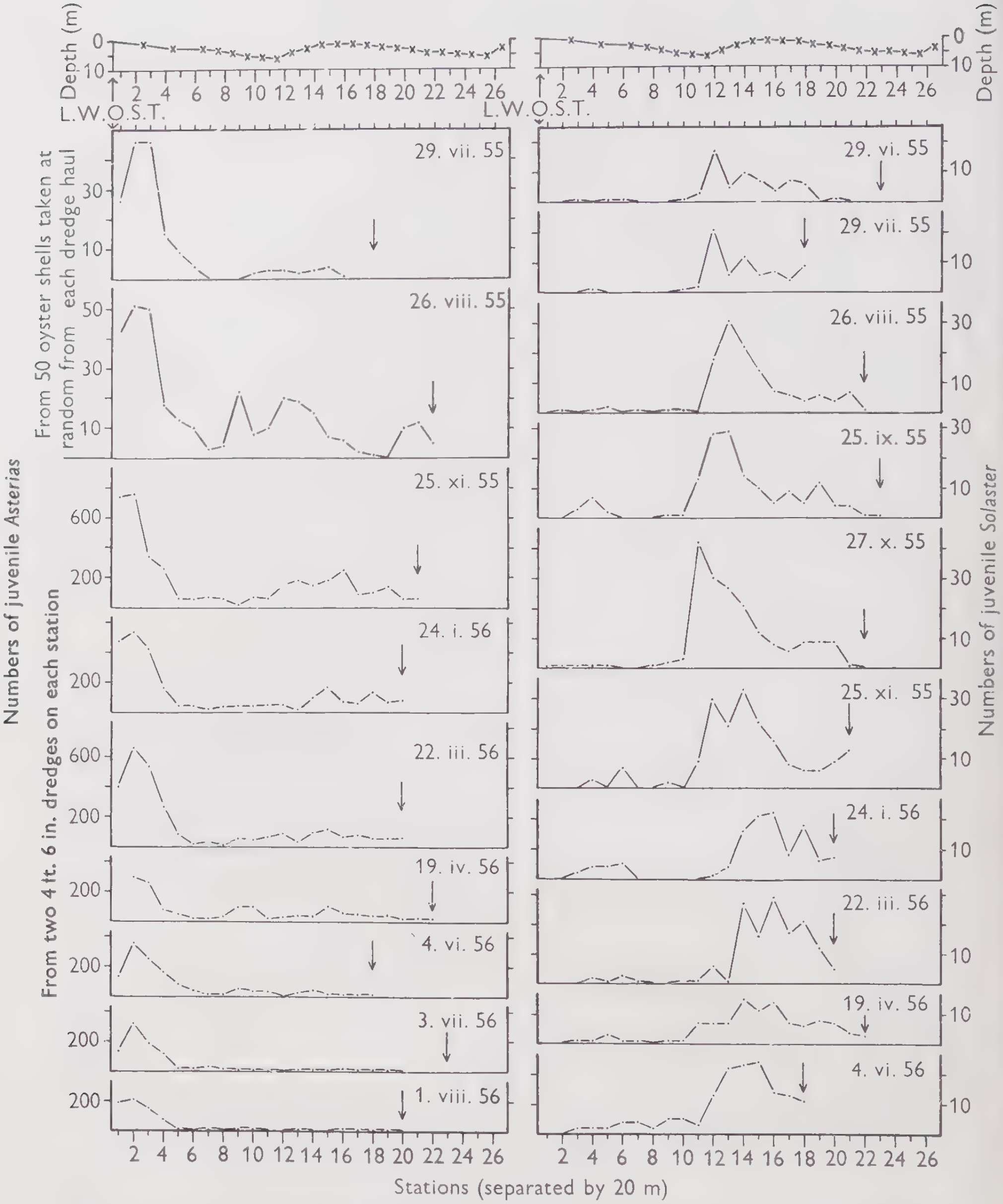

Fig. 3. Numbers of juvenile *Asterias* and *Solaster* taken using two 4 ft. 6 in. power dredges in parallel dredge hauls across the River Crouch at the Southward Laying 1955–56, commencing 10 m from L.W.O.S.T. on the north shore. On 29 July and 26 August 1955 *Asterias* were counted from only 50 shells in each dredge haul. From 25 November onwards, the total number in each dredge haul has been represented. Sections across the river showing mean depths are given. The final dredge station on each date is indicated by an arrow.

There was in 1955, therefore, considerably more overlapping of the juveniles of the two starfishes than of the adult populations. Young *Asterias* settled in maximum density inshore on cultivated oyster ground, but settling also occurred farther offshore in the *Solaster* zone. Young *Solaster* settled mainly in mid-river in the region of greatest abundance of adults, suggesting perhaps a gregariousness of settling near the parents as in certain other invertebrates (Cole & Knight-Jones, 1949, in oysters; Knight-Jones & Stevenson, 1950, in *Elminius*). Both young and adult *Solaster* have been observed devouring *Asterias*, particularly juveniles, and this, coupled with the discrete settling behaviour of *Solaster*, is believed to be responsible for the no more than slight overlapping of the two adult populations. *Asterias* is capable in a lesser capacity, of devouring young *Solaster*. Although there was such an enormous settlement of *Asterias* on the Southward Laying in 1955, the numbers taken in dredge hauls decreased steadily—from over 2700 on Station 2 on 23 September 1955 to only 44 on 15 October 1956. The distribution at the Southward Laying showed that this decrease was occurring on all stations, that is, it was not due to an inshore or offshore movement. A certain amount of migration of juveniles to neighbouring grounds might have occurred, but conditions there were no different from the Southward Laying. It is believed that the great decrease in the numbers was due to a heavy mortality caused by competition and cannibalism, resulting from a prolonged shortage of natural food and predation by *Solaster*. Galtsoff & Loosanoff (1939), in America, observed that an abundant set of *Asterias forbesi* Desor, greatly decreased within 1 month after settling, and suggested that cannibalism, observed frequently between young starfish in the laboratory, was responsible. It is significant that, before the settlement of young *Asterias* in 1955, shells and oysters at the Southward Laying were densely covered by barnacles, but by the end of August 1955 it was impossible to find a live barnacle there. Until the end of 1956, although barnacle settlement was elsewhere normal, the Southward Laying was still free. It is believed that the juvenile *Asterias* devoured the barnacles, giving them no chance to grow to recognizable size. The absence of freshly settled stages of *Asterias* in 1956 may have been a direct consequence of the lack of young barnacles required as food. By mid-1956, the distribution of the young of the two species of starfish resembled that of the adult populations (Figs. 2, 3). It is interesting to note that following the reduction of the numbers of *Asterias* to a normal level, barnacles reappeared in large numbers on the Southward Laying in 1957.

POPULATION DENSITY

Two methods were employed to estimate the density of young *Asterias* on the Southward Laying. These had been used previously with some success for determining the population density of *Urosalpinx* (Hancock, in press), and comprised (*a*) marking and recapture experiments, and (*b*) a survey using a $\frac{1}{10}$ m^2 van Veen grab. Loosanoff (1937, 1953) described the marking of starfishes from the Atlantic coast of America with Nile Blue Sulphate, and Vernon (1937) used Neutral Red. Feder (1955) used both these methods with success on Pacific coast starfishes, and found that plastic disc tags attached by stainless steel were useful, but very liable to cause injury or loss of the tagged arms. No doubt the proportion surviving uninjured would be useful in migration or growth studies, but the method would be less reliable for recapture data.

Employing method (*a*), an area of oyster ground 20 m wide was marked out with buoys, its inner edge being parallel to, and 20 m below, the shore at L.W.O.S.T. on the Southward Laying (Fig. 1, area D–B). Dredge hauls were made on this area on 23 September 1955, parallel to the shore, and 1000 young *Asterias* were marked and returned, to be searched for by subsequent dredging. Marking was done by diluting a stock solution of Nile Blue Sulphate about ten times with sea water. This was sufficient to dye a large number of *Asterias* immersed in it in 5–10 min. The normal reddish brown of the aboral surface became darker and bluish-green in colour. The blue coloration showed up most clearly on the parts normally white, such as the surface of spines, and the oral surface. The blue stain of Nile Blue Sulphate was retained by marked starfish until at least 10 months after marking. A series of young and old dyed *Asterias* were retained in tanks in the laboratory for several months with no significant mortality.

Young *Asterias* were capable of quite rapid movement and for this reason only an hour or two was allowed for mixing with the normal population before redredging. Dredging, marking and redredging was continued on 12 and 14 October. The first group marked on 23 September totalled 1000, and on 12 October a second group of 1450 and a final group of 2165 were dyed making a total of 4615. Bailey's (1951) modification of the 'Lincoln Index' was applied to the numbers recaptured on each date. This is: $\check{x}=a(n+1)/(r+1)$, where $\check{x}$ is the total population in the marked area, a is the number of individuals marked, n is the size of a random sample containing r marked individuals. The total number of recaptured starfish was 365, compared with 16,903 unmarked. From calculations based on marking of the three groups (Table 2), the average figure for young *Asterias* per m^2 over the total area of 2500 m^2 used for recaptures was found to be 131. Uneven distribution of marked starfish over the area may account for the large variation between 28 and 296 per m^2 shown in Table 2.

The sorting of several thousand small starfish from the contents of a dredge is tedious and lengthy, and it was felt that a grab survey might be more accurate and would take less time. Grabbing could not be done until a month later, on 10 November, so that the results cannot be compared strictly with those from the recapture experiment described above. An area of oyster ground below L.W.O.S.T. was divided up with buoys to include the area used in the recapture experiment (Fig. 1). Ten grab hauls ($\frac{1}{10}$ m^2) were made at random in each subarea (Fig. 1, A–E), so that their total represents the density per square metre for each station. The results showed great variation between single grab samples, from 0–15 on one station (Fig. 1, C). The bottom deposits were found to be variable, and the absence of *Asterias* could usually be related to clay or mud with no shells. A maximum of 59 per m^2 was found on Station C, with an average of 37 per m^2 for the whole area. Station D had 18, and Station B, 27 starfish per m^2, giving an average of 23 for the area D–B used in the recapture experiment.

The mid-level area D–B (Fig. 1) used for the recapture experiment was re-examined by a further 10 grab hauls intended to confirm the original results (Fig. 1). This time, however a total of 65 starfish per m^2 was obtained, compared with the previous average of 23 per m^2. The variation from 1 to 22 in individual grab hauls (Fig. 1) again emphasizes the patchiness of *Asterias*, and shows that a much larger number of grab hauls would be required to give more significant results.

TABLE 2. RECAPTURES OF MARKED *ASTERIAS* FROM DREDGE HAULS OVER STATION 2 (D–B)

(Each haul with two 4 ft. 6 in. dredges.)

Date of marking	Total number marked (a)	Date of recatpure	Number of recaptures (r)	Number unmarked ($n-r$)	Calculated total in the area (2500 m^2) $\frac{a(n+1)}{(r+1)}$	Average per m^2	Average (per m^2) for group
23 Sept. 55	1000	23 Sept. 55	7	2717	340,625	136	167
23 Sept. 55	1000	26 Sept. 55	3	2481	621,250	249	
23 Sept. 55	1000	12 Oct. 55	6	1997	286,286	115	
12 Oct. 55, (x)	2450	12 Oct. 55	8	1718	470,128	188	166
12 Oct. 55, (x)	2450	12 Oct. 55	1	290	357,700	143	
12 Oct. 55, (y)	4615	14 Oct. 55	30	636	99,297	40	95
12 Oct. 55, (y)	4615	14 Oct. 55	126	1770	68,934	28	
12 Oct. 55, (y)	4615	14 Oct. 55	10	1754	740,498	296	
12 Oct. 55, (y)	4615	14 Oct. 55	33	1511	209,711	84	
12 Oct. 55, (y)	4615	14 Oct. 55	141	2029	70,558	28	

Final average per 2500 m^2 for all recaptures = 326,499.
Average per m^2 for all recaptures = 131.
(x), (y): separate batches marked on the same day.

The figure of 37 per m^2, representing the average of the two series of grab hauls on Stations D–B, differs substantially from that of 131 obtained from recapture data from the same area, but it must be remembered that grab sampling was delayed for one month. Dredge survey results (Fig. 3) indicated that the density of young *Asterias* over the whole Southward Laying area was decreasing during this period; on 25 November 1955, for example, less than 800 were obtained per haul with two dredges on Station 2 (Fig. 1, over D–B) compared with the usual catch of nearly 2000 before the recapture experiment. The largest number obtained in two 4 ft. 6 in. wide dredges dragged over 125 m on the same area was 2724 on 23 September 1955 on Station 2, which, when related to the area covered by the dredges, represents only 8 per m^2, and demonstrates the low efficiency of dredges for catching small starfish.

It is evident that, by either method, the estimation of density of a large population with such irregular distribution, even over a small area, can be based with confidence only on intensive sampling.

FEEDING

LABORATORY EXPERIMENTS

It was demonstrated in laboratory experiments (Hancock, 1955) that, although *Asterias* occasionally ate spat and adult oysters, the greater part of its food was composed of animals in competition with the oyster. The smaller sizes of *Asterias* ate large numbers of barnacles, with occasional spat of oysters and *Crepidula*. The larger ones occasionally ate oysters and oyster spat, but almost always exhibited a preference for mussels, and, in the absence of these, for *Crepidula*, and sometimes even for *Urosalpinx*.

To assess the effects of the very large number of juvenile *Asterias* which settled on the cultivated part of the oyster ground at the Southward Laying during 1955, further studies were made of the feeding behaviour of young starfish. These experiments also provided valuable information on growth.

Experiment 1

Ten *Asterias* of maximum radius 1–8 mm, were confined with an unlimited supply of barnacles, mainly *Elminius modestus* and *Balanus* spp. From 6 to 15 August 1955, 954 barnacles were devoured, representing an average of 10·6 barnacles per *Asterias* per day. The maximum feeding rate was 15·2 barnacles per day.

Experiment 2

From 9–15 August 1955, ten *Asterias* of maximum radius 1–8 mm were confined with shells bearing oyster spat, *Crepidula* spat and barnacles. During the first 3 days barnacles were eaten from all the shells, but no oyster spat, which were on the same shells, were taken. Towards the end of the experiment some oyster spat were taken, but nearly all of the barnacles had been destroyed. A total of 582 barnacles and 11 oyster spat (6 of 2 mm, 4 of 3 mm, and 1 of 7 mm average diameter), but no *Crepidula* spat, were eaten in 6 days. The *Asterias* finally measured 3·7–10·9 mm.

Experiment 3

Thirty *Asterias*, 12–13 mm max. radius, were isolated with *Crepidula* spat. In 7 days, 10–17 September 1955, only 5 *Crepidula* (7–9 mm in length) were eaten. When ten of these *Asterias* were transferred to a tank containing mussel spat, these were attacked immediately and the feeding rate greatly increased. The remaining 20 *Asterias* were retained with *Crepidula* spat, but the feeding rate continued to be low. From

10 September to 19 November 1955, only 26 *Crepidula* spat (4–13 mm in length) were eaten. The *Asterias* finally measured 8–12 mm. Only fourteen of the *Asterias* remained on 19 November 1955, the others having been the victims of cannibalism.

Experiment 4

Twenty *Asterias*, 12–13 mm, were isolated with oyster spat. Between 10 September and 2 October 1955, only 6 spat (4–8 mm diameter) were eaten. When barnacles were added on 12 October, the feeding rate increased, and, although 2566 barnacles were devoured between 12 October and 31 December 1955, no further oyster spat were taken. This experiment showed a maximum feeding rate of 5 barnacles per *Asterias* per day. The measurements of the *Asterias* were as follows:

Date (1955)	Maximum radius (mm)	Food
10 Sept.	12–13	—
12 Oct.	9–12	Oyster spat
4 Nov.	9–14	Barnacles
31 Dec.	9–19	Barnacles

Experiment 5

17 September 1955. Ten *Asterias* from Expt. 3 (12–13 mm) were confined with many mussel spat. Initially the feeding rate was high, but, as the size range of the mussel spat increased through growth, they gradually became too large for small starfish to open, and, by the beginning of December, when the mussels were all about 20 mm in length, feeding virtually ceased. When the mussels were replaced with barnacles, however, a high feeding rate was resumed.

Date (1955)	Size range of mussels (mm)	Number eaten by 10 *Asterias*	Number per *Asterias* per day
17–30 Sept.	3–10	165	1·3
1–31 Oct.	2–15	269	0·9
1–30 Nov.	3–24	84	0·3
1–7 Dec.	20–23	3	0·04
7–31 Dec.	Barnacles	480 barnacles	2 barnacles

The measurements of the starfish were as follows:

Date (1955)	Maximum radius (mm)	Food
10–17 Sept.	12–13	*Crepidula* spat
17 Sept.–12 Oct.	10–17	*Mytilus* spat
12 Oct.–14 Nov.	15–24	*Mytilus* spat
14 Nov.–31 Dec.	16–29	*Mytilus* spat and barnacles

Experiment 6

Eleven *Asterias* (all of maximum radius 20 mm) were offered equal numbers of *Crepidula* and oyster spat. Between 1 January and 29 March 1956, 32 oyster spat and 42 *Crepidula* spat were eaten. By the end of March the range of maximum radius length had decreased from 20 mm to 8–16 mm. Oyster spat eaten had a maximum diameter range of 8–25 mm and *Crepidula* 5–22 mm. The growth of these starfish is discussed below.

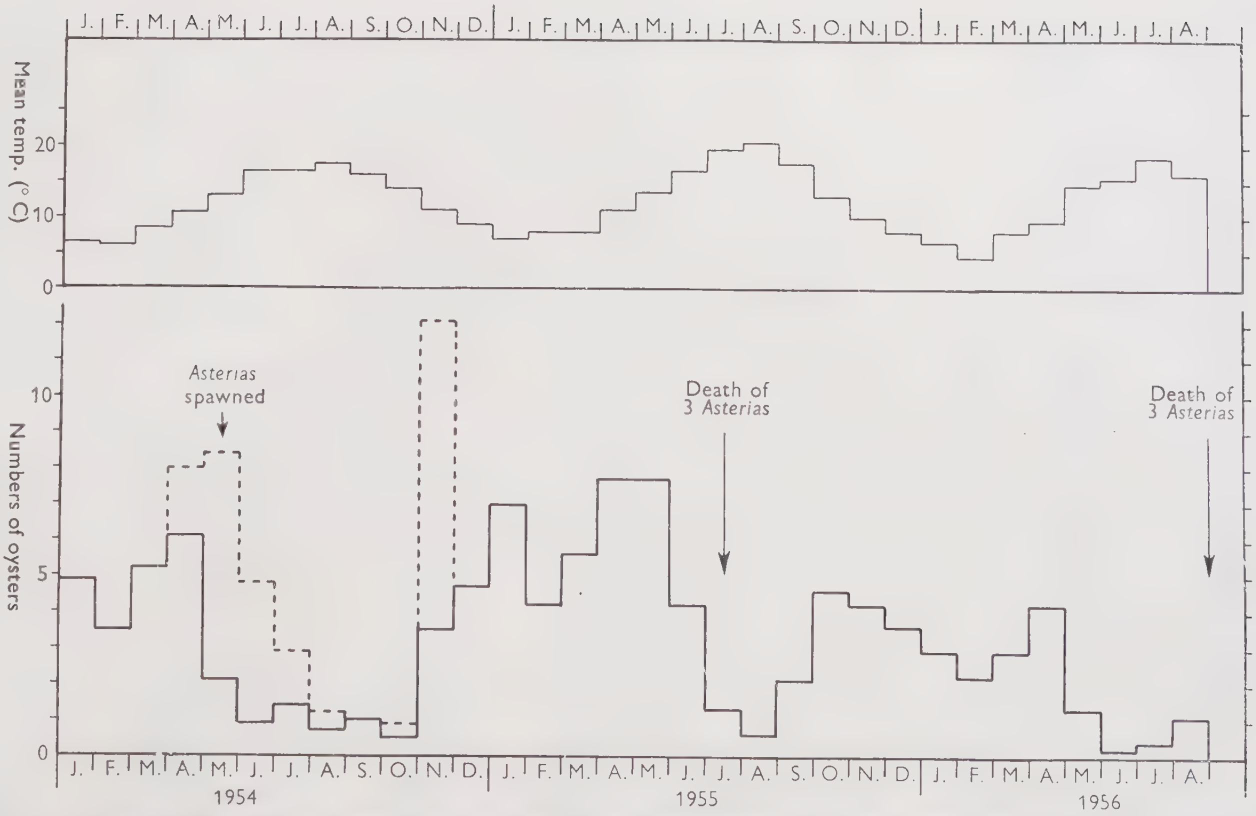

Fig. 4. Average numbers of adult oysters eaten per week by four large *Asterias* during each month from January 1954 to August 1956. From April to November 1954, *Crepidula* were offered as an alternative, and the numbers eaten in addition to oysters have been shown by a broken line. Mean monthly temperatures are also given.

Experiment 7

Five *Asterias* (maximum radius 20–30 mm) were confined with roughly equal quantities of barnacles and mussel spat. Between 26 September and 22 October 1956, 665 barnacles and 293 mussels were eaten; between 23 October and 19 November a further 262 barnacles and 636 mussels. Mussel spat occur infrequently on the Southward Laying. This experiment may provide an instance of starfish attacking, at first, familiar food, and then turning their attention to an unfamiliar alternative, which they evidently liked. Alternatively, food habits may change with growth.

Experiment 8

On 10 September 1955 a medium-sized *Solaster* (31 mm maximum radius) was confined with 20 *Asterias* (14–15 mm) and no other food. The *Solaster* needed from 10 September to 12 November 1955 to devour all the *Asterias*. During this time the size measurements of the starved *Asterias* decreased.

Experiment 9

On 10 September 1955 two small *Solaster* (maximum radius, 10–11 mm) were isolated with 20 *Asterias* (10–11 mm) and no other food. When this experiment was ended on 19 November, 12 *Asterias*. remained, though greatly decreased in size, and one *Solaster* had been eaten by the *Asterias*. The size-range of the starved *Asterias* decreased as follows: 10 September, 10–11 mm; 12 October, 8–11 mm; 14 November, 6–10 mm.

Experiment 10

The experiment described previously (Hancock, 1955, Expt. 1, p. 317), in which four large adult *Asterias* (maximum radius 140 mm) were fed on adult oysters, was continued to try to demonstrate changes in feeding rate at different times of the year. The results are shown in Fig. 4. They are slightly complicated by the addition of a number of *Crepidula* on 21 April 1954, and their removal on 13 November 1954. The numbers of *Crepidula* eaten are shown in Fig. 4, and can be seen to exceed the numbers of oysters eaten by the *Asterias*, even though the latter had been conditioned to feeding on oysters for several months. Spawning of the *Asterias* took place in the laboratory during May, and feeding reached a maximum in April–May of each year, but the rate dropped soon after spawning and reached a minimum in August during the period of highest water temperatures. Steady feeding took place throughout the winter, with a slight drop in feeding rate each February, usually corresponding with the lowest monthly average of mean daily temperatures. The optimum feeding temperature appears to be between 10 and 13° C. and occurs in April–May; above this temperature the feeding rate declines. It must be remembered, however, that the lowered feeding rate after April–May may have been associated with post-spawning effects.

Galtsoff & Loosanoff (1939) kept starfish (*Asterias forbesi*) in tanks in the United States, and found that during the pre-spawning period, from the end of May until July, at temperatures from 11·0 to 14·5° C., the majority were indifferent to food. Soon after the completion of spawning starfish became exceedingly voracious, and continued to be so until the onset of cold weather. With the vernal rise of temperature, starfish became more and more activated but ceased eating with the approach of the breeding period.

FIELD OBSERVATIONS

Throughout the surveys of the Southward Laying, records were kept of observations of feeding by *Asterias* and *Solaster*. *Asterias* was only occasionally taken when feeding, but the food of *Solaster*, which can ingest most food without pre-digesting it, was more easily recorded. *Asterias* was observed on four occasions devouring an oyster, and sixteen times eating *Crepidula*. The records of the food of *Solaster* were: *Asterias* 64, *Crepidula* 9, *Alcyonidium* 15, *Gibbula* 11, and the anemone *Diadumene* 7. The *Solaster* which were feeding on *Asterias* varied in size from 13 to 85 mm maximum radius, and it was not until late September 1955 that the incidence of feeding on *Asterias* became frequent, increasing to a maximum in March 1956. Before September *Solaster* was observed feeding mainly on other animals, this change no doubt being associated with the settlement of young *Asterias* on the offshore part of the Southward Laying inhabited by *Solaster*. Two *Solaster* (62 and 66 mm maximum radius) were found eating smaller ones of the same species. One *Solaster* of only 14 mm maximum radius was found to have enveloped a *Gibbula* of 10 mm shell diameter. Feeding on *Alcyonidium* was mostly confined to the summer months when this bryozoan was in the active phase. These results agree with the observations of Fulton (1895), who found arms of *Asterias* protruding from the mouths of many specimens of *Solaster* dredged on oyster beds in the Firth of Forth.

Adult *Asterias* taken in dredge hauls in the River Swale, Kent, in December 1956, were observed to be feeding both on adult mussels and *Crepidula* which existed together there in abundance.

PRACTICAL CONSIDERATIONS

The laboratory feeding experiments showed that young *Asterias* ate large numbers of barnacles and young mussels, on which they maintained a steady growth. Oyster spat and *Crepidula* spat were much less acceptable, and such a diet alone was inadequate for growth, with actual reduction in size often resulting. Subsequent growth of the prey under these conditions made it less liable to attack by *Asterias*. Smith (1940) found that the rate of growth of *A. vulgaris* in eastern Canada varied with the kind and abundance of food. Mussels provided a much more favourable food for growth than oysters.

The spatfall of oysters at the Southward Laying is rarely intense, and *Urosalpinx* regularly destroys nearly all those present (Hancock, 1954), so that as juveniles the starfish would normally not be a serious problem there. In fact some benefit must have resulted from the activities of the *Asterias* in keeping down the barnacles, which are the most important competitors of settling oysters for space and food (Waugh & Ansell, 1956). A study of the seasonal feeding rate of adult *Asterias* showed that the best time to inspect for damage to highly cultivated oyster grounds is in the spring of the year before the starfish spawn. There has been no fresh evidence that *Asterias* is a serious pest of oysters under the conditions obtaining in Essex rivers, and both in the laboratory and in nature the starfish have continued to feed preferentially on competitors of the oyster.

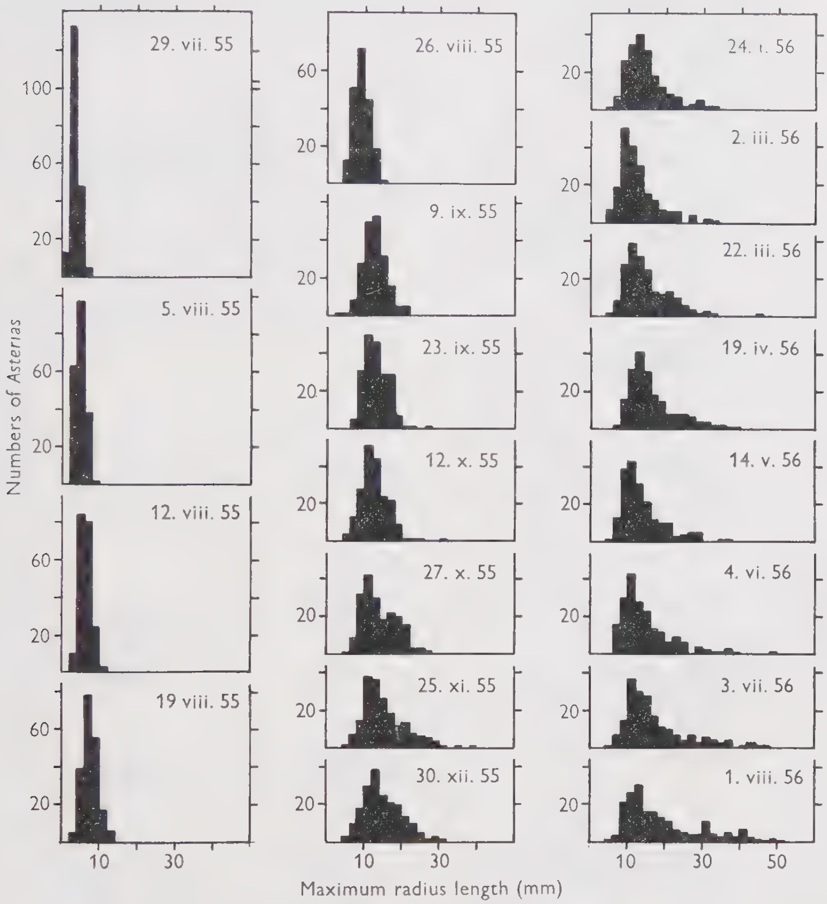

Fig. 5. Histograms showing the size distribution of young *Asterias* collected in dredge surveys from the Southward Laying 1955–56. Each sample was composed of 200 individuals. The radius lengths have been grouped at 2 mm intervals.

GROWTH

The study of population histograms does not give a clear indication of growth under natural conditions unless (1) settlement of any year group takes place over a restricted period, and (2) a large number of the form being studied settle in a particular year, so that the progress of this year group can be traced

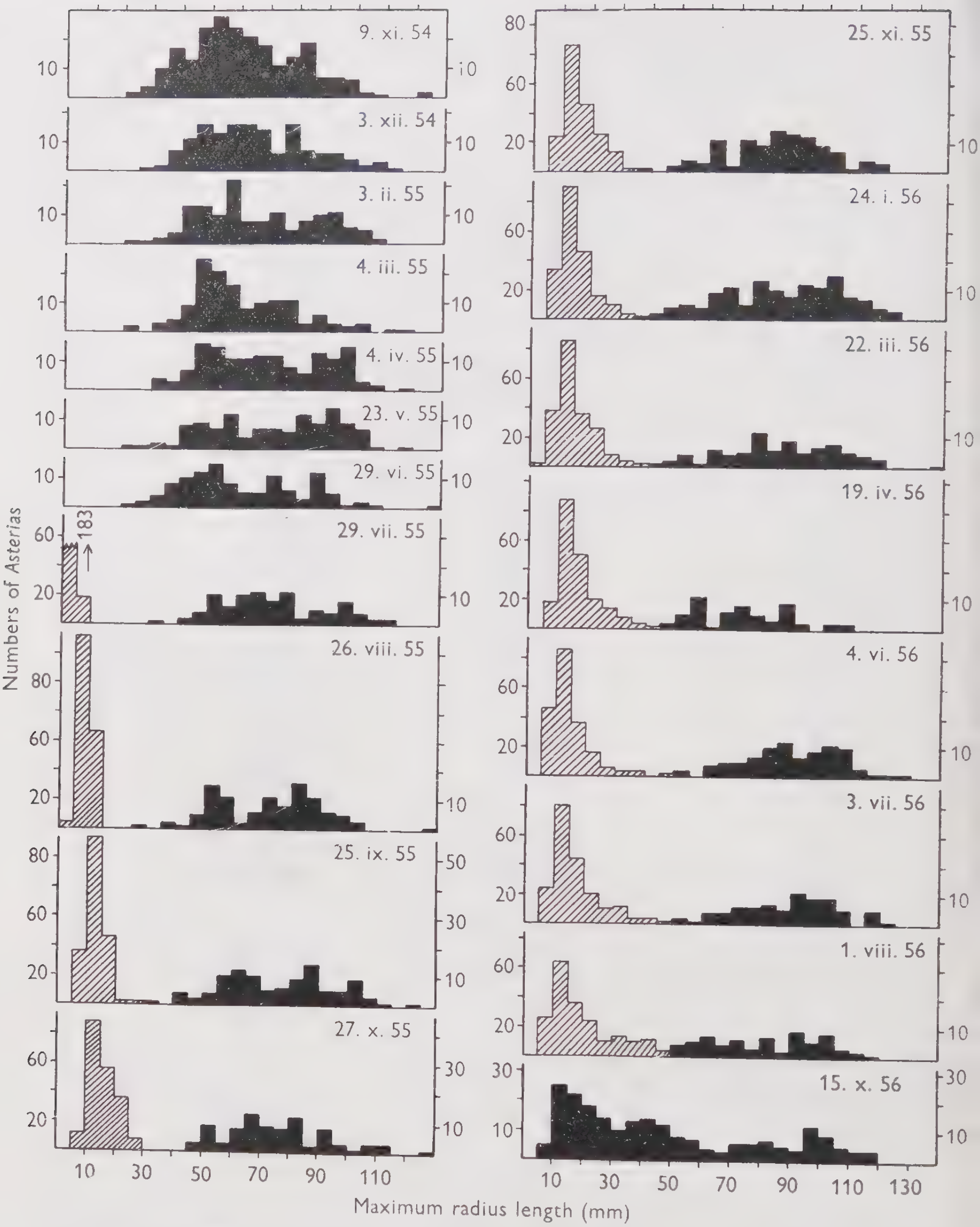

Fig. 6. Histograms showing the size distribution of young (shaded) and adult *Asterias* (blackened) collected in dredge surveys from the Southward Laying, 1954–56. Samples of 200 juveniles were measured, but all adult *Asterias* taken in the dredge surveys are shown. On 15 October 1956 all of both juveniles and adults taken were measured. The radius lengths have been grouped at 5 mm intervals, using different ordinate scales for adult and young starfish.

in consecutive histograms. It was fortunate that heavy settlements of both *Asterias* and *Solaster* occurred in 1955.

Histograms have been plotted at intervals of 2 mm for measurements of the maximum radius of arm to show the growth within the 1955 year group (Figs. 5, 7) and at 5 mm intervals to show the relationship of the year groups and their progress along the histograms (Figs. 6, 8). The *Asterias* population was sampled in two ways. First, the resident population was sampled,

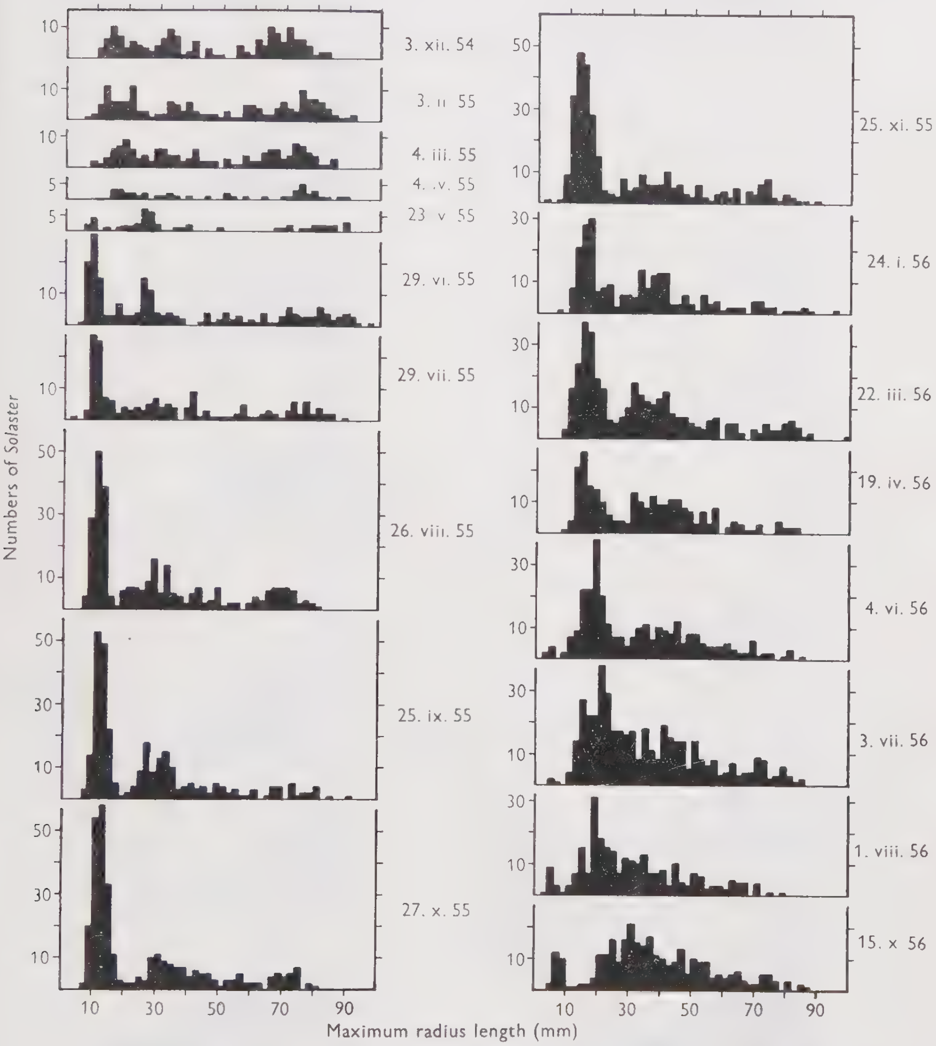

Fig. 7. Histograms showing the size distribution of *Solaster* collected in dredge surveys from the Southward Laying 1954–56. The radius lengths have been grouped at 2 mm intervals.

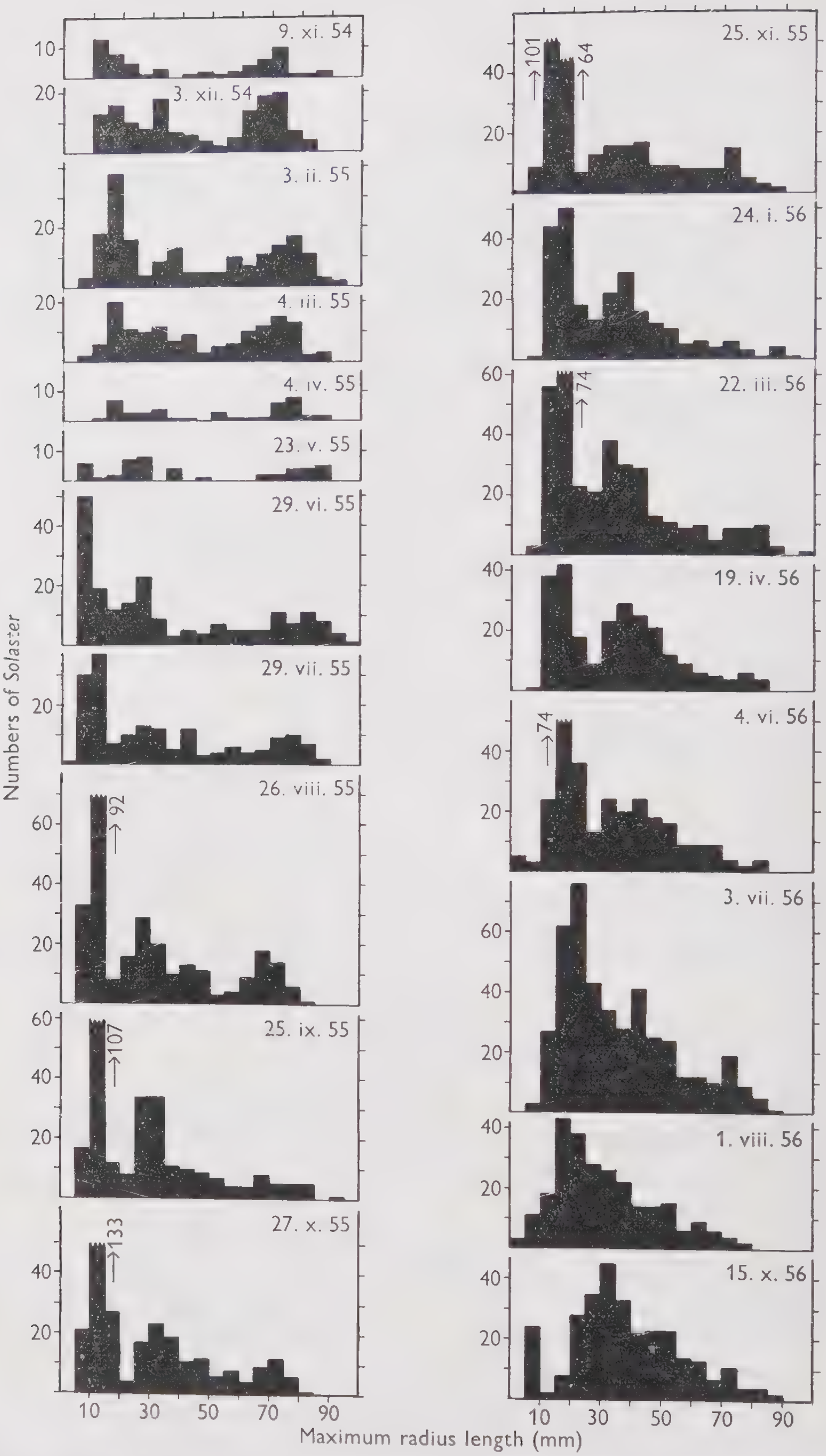

Fig. 8. Histograms showing the size distribution of *Solaster* collected in dredge surveys from the Southward Laying 1954–56. The radius lengths have been grouped at 5 mm intervals.

beginning on 9 November 1954, by measuring all *Asterias* collected in two dredge hauls over the stations examined on a particular date (Fig. 2). The settlement of 1955 0-group individuals was first noted on 29 July 1955, when the juveniles had a size range of 1–7 mm and could readily be distinguished from the older year groups in the population. Measurement of all of the enormous quantity of juveniles collected was impracticable, so that although the remainder of the population was measured as before, only a randomly selected sample of 0-group individuals from Station 2 was measured.

TABLE 3. GROWTH OF JUVENILE *ASTERIAS* AND *SOLASTER* AT SOUTHWARD LAYING, RIVER CROUCH

	Asterias			*Solaster*		
Date of collection	No. in sample	Mean length of sample (mm)	Standard error of mean	No. in sample	Mean length of sample (mm)	Standard error of mean
29. vi. 55	—	—	—	69	8·5	0·21
29. vii. 55	200	2·9	0·08	71	10·2	0·29
5. viii. 55	200	4·3	0·10	—	—	—
12. viii. 55	200	5·7	0·11	—	—	—
19. viii. 55	200	7·0	0·14	—	—	—
26. viii. 55	200	8·6	0·15	127	10·7	0·16
9. ix. 55	200	12·1	0·22	—	—	—
23. ix. 55	210	12·2	0·23	—	—	—
25. ix. 55	—	—	—	149	11·8	0·19
12. x. 55	200	12·1	0·27	—	—	—
27. x. 55	200	15·4	0·33	184	12·3	0·18
25. xi. 55	210	15·0	0·40	189	13·9	0·24
30. xii. 55	200	14·4	0·36	—	—	—
24. i. 56	246	14·2	0·34	114	15·8	0·33
2. iii. 56	241	11·8	0·32	—	—	—
22. iii. 56	473	14·3	0·26	160	15·8	0·28
19. iv. 56	278	15·9	0·38	105	16·6	0·40
14. v. 56	253	13·2	0·35	—	—	—
4. vi. 56	275	13·8	0·39	149	18·2	0·35
3. vii. 56	215	16·6	0·57	—	—	—
1. viii. 56	216	19·2	0·72	—	—	—

This sample was used to determine the standard error of the mean (Table 3). The measurements of only the first 200 individuals in each sample, however, were used for the comparative histograms (Figs. 5, 6). For this reason, in Fig. 6, the 0-group is shown on a different scale from that of the main population and is distinctly shaded. In fact, the 0-group *Asterias* represented a far larger proportion of the population than shown, and some idea of their density can be obtained by reference to Fig. 3. This method of subsampling was continued until 15 October 1956, when this year group had become so reduced that all the *Asterias* taken in the dredges could be measured. The numbers of young *Solaster* were never so great that they could not all be measured with the remainder of the population.

The mean size of each sample of 1955 0-group *Asterias* and *Solaster* (Table 3) has been plotted in fig. 9. *Solaster* showed a regular growth rate except between 24 January and 22 March 1956. The average growth between

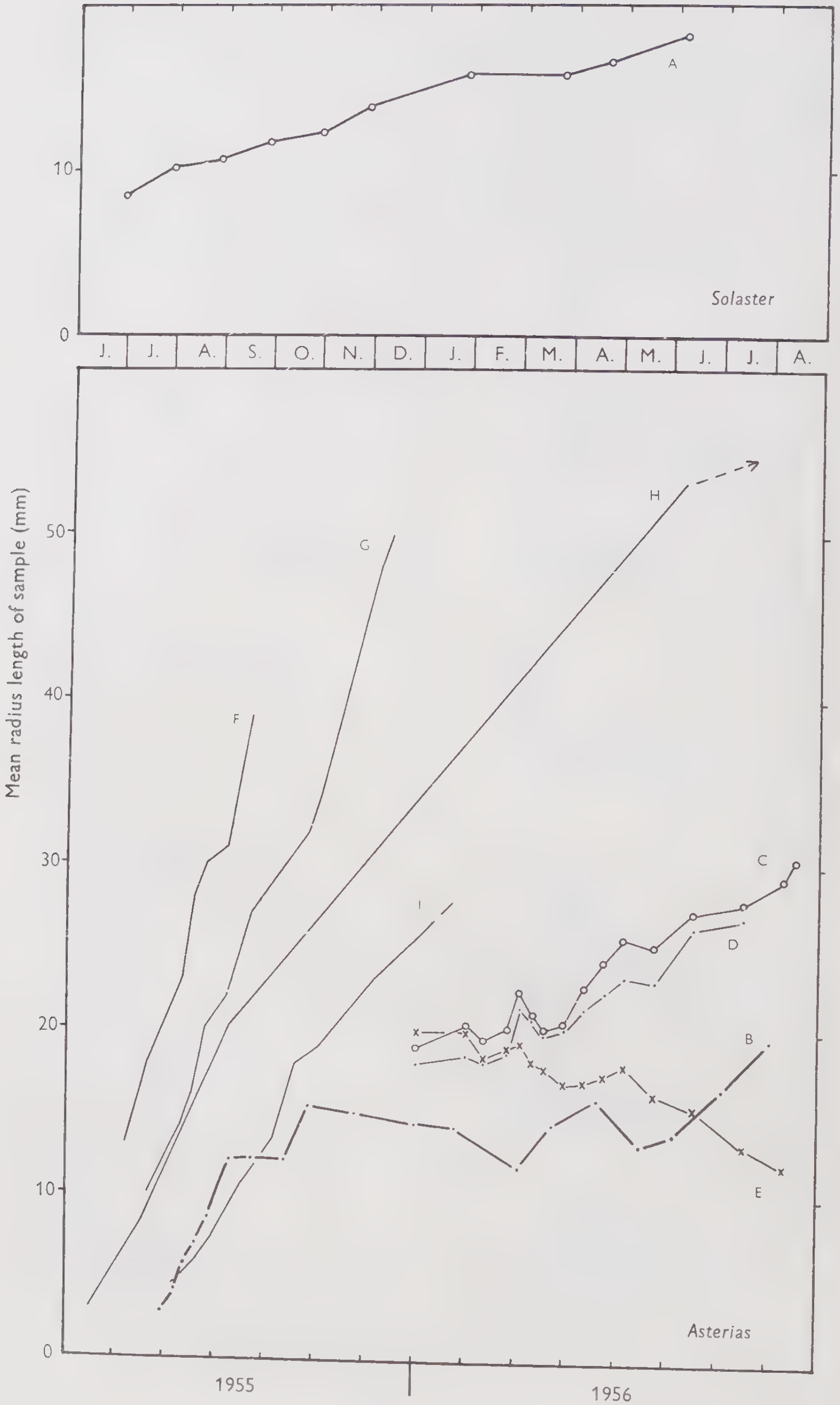
A
10
0
Solaster
J. J. A. S. O. N. D. J. F. M. A. M. J. J. A.
Mean radius length of sample (mm)
50
40
30
20
10
0
G
H
F
I
C
D
B
E
Asterias
1955
1956

Fig. 9

29 June 1955 and 4 June 1956 has been calculated as 0·028 mm per day, with a maximum rate of 0·056 mm per day between 27 October and 25 November 1955.

A 1956 settlement of *Solaster* was first observed on 4 June, but the juveniles were less abundant than in 1955, and on 15 October 1956 their mean size was only 6·8 mm, compared with 12·3 mm for the 1955 0-group on 27 October 1955. This may have been due to a later settlement in 1956, or to the negligible crop of *Asterias* in 1956, whose young form the main food of *Solaster*. It is possible to trace the progression of the 1954 0-group *Solaster* from November 1954. A comparison of the year groups on 3 February 1955 and 24 January 1956 (Fig. 8), shows the first two modes in a similar position, indicating that in 1954 and 1955 similar conditions of growth and settlement prevailed. On 4 June, when the 1956 settlement was first observed, the 1955 set of *Solaster*, at just over 1 year old, had a mean size of 18·1 mm while the 1954 set averaged about 40 mm. From Fig. 8, it appears that in October the modes of the first few year groups are separated by regular intervals of about 20 mm; they occurred at about 10, 30–35, 50, and 70–75 mm respectively. Subsequent year groups were not clearly defined.

To state precisely the growth pattern of *Asterias* on the Southward Laying is more difficult. Newly settled juveniles were first detected in 1955 on 29 July at a mean radius of 2·9 mm. Regular rapid growth, averaging 0·22 mm per day, was recorded until 9 September (mean radius 12·1 mm), but then a check occurred. Mean size increased again between 12 and 27 October, but then decreased sharply until 2 March 1956. Reference to Fig. 5 shows that the decrease in mean size resulted from two processes; first, the range of size was increased by the slight growth of a small proportion of larger individuals, and, secondly, the mean size decreased. This is less surprising than may at first appear, in view of the shrinkage in radius length of starving *Asterias* noted in a previous section (see also Vevers, 1949). It is known that very small food organisms, e.g. barnacles, are necessary for the normal feeding and growth of young starfish. It is believed that the shortage of natural food was responsible for the enormous decline in numbers of young *Asterias* (Fig. 3) and for the reduction in the mean sizes of samples by actual shrinkage of individuals.

Growth of part of the juvenile population continued steadily from 2 March

Legend to Fig. 9

Fig. 9. Graphs showing the growth of young *Asterias* and *Solaster*. A: mean radius lengths of samples of young *Solaster* from the Southward Laying. B: mean radius lengths of samples of young *Asterias* from the Southward Laying (see Table 3). C–E: mean radius lengths of young *Asterias* kept well fed (C, D) and starved (E) in the laboratory (see Table 4). The results of other workers are given for comparison, showing the correct calendar months for their readings. F, G: radius lengths of two typical well-fed individuals of *Asterias*, B8 and B9, kept in the laboratory by Vevers (1949). H: average radius of the arms of an *Asterias* kept in the laboratory by Bull (1934). I: mean radius lengths of samples of *Asterias* from Millport measured by Barnes & Powell (1951).

to 19 April 1956, while the mode still remained at 10–15 mm (Fig. 5). Then, after another setback in mean size, growth of juveniles continued to give the bimodal size-distribution shown for 15 October 1956 in Fig. 6. The first mode, at 10–15 mm, lies in exactly the same position as it was a year previously on 27 October 1955. Unless one knew that 1956 settlement of *Asterias* had failed, the bimodal year group shown on the October 1956 histogram might be attributed to two normal year groups, instead of to the effect of abnormal growth conditions.

Bull (1934) showed that in three *Asterias* kept in the laboratory, first spawning occurred simultaneously when two were 5 years old and the third was 6 years old. Their average size was 114 mm. It seems unlikely therefore that the checks in growth observed during the first 2 years of the present observations were associated with spawning. Galtsoff & Loosanoff (1939), however, found that in America young *Asterias forbesi* which grew rapidly were sexually mature by the end of the first year, whereas small, slowly growing animals did not mature until 2 years of age.

TABLE 4. GROWTH OF WELL-FED AND STARVED JUVENILE *ASTERIAS* IN THE LABORATORY

(12 *Asterias* in each group.)

	Starved *Asterias*		Well fed *Asterias*			
Date of measurement	Mean size of group	Range	Mean size of group	Range	Mean size of group	Range
1. i. 56	20	20	19	19	18	18
1. ii. 56	20	18–22	20·3	18–26	18·5	17–20
11. ii. 56	18·3	16–21	19·5	17–25	18·2	17–20
25. ii. 56	19·0	16–22	20·2	18–25	18·8	17–23
3. iii. 56	19·3	16–21	22·4	20–25	21·5	20–24
10. iii. 56	18·2	16–20	21·0	19–24	20·8	17–23
17. iii. 56	17·8	15–19	20·1	18–24	19·8	17–22
29. iii. 56	16·8	14–19	20·4	19–22	20·2	17–23
11. iv. 56	17·0	14–19	22·7	21–24*	21·4	15–25
23. iv. 56	17·4	16–19*	24·3	22–26	22·3	19–26
5. v. 56	18·0	17–20	25·7	23–27*	23·3	18–27
23. v. 56	16·2	15–18	25·2	23–28	23·0	20–28
16. vi. 56	15·5	13–18	27·3	25–29	26·3	21–32*
16. vii. 56	13·2	11–15	27·8	23–31	26·9	21–33**
10. viii. 56	12·0	11–14	29·3	24–33	—	—
17. viii. 56	—	—	30·5	28–33	—	—

* 1 *Asterias* died.

Experiments were set up to compare the growth of regularly fed and starved starfish. On 1 January 1956, a group of twelve 20 mm *Asterias* was confined with oyster and *Crepidula* spat; from previous experience, one would regard these as having virtually starved. Separate groups of twelve 19 mm and twelve 18 mm *Asterias* were confined with an excess of live barnacles. The starfish were measured regularly, along a selected radius, until July–August 1956, when the original 20 mm group had reached 11–14 mm, the 19 mm group 28–33 mm, and the 18 mm. group 21–33 mm (Table 4). The mean sizes of each group on the dates of measurement are shown in Fig. 9, and it is interesting to note that decreases in mean size were recorded, even for regularly fed *Asterias*, in February, March and May 1956. A sharp decrease, followed by an increase in size, was recorded in February among both the starved 20 mm *Asterias* in the laboratory and the natural population at the Southward Laying; this corresponded with the minimum water temperatures recorded in the winter of 1955–56. The May decrease in mean size of well-fed laboratory individuals was evident in the Southward Laying population, and was also shown to a certain extent by a slightly accelerated decrease in the size of starved laboratory specimens. The author is indebted to Mr S. J. Holt of F.A.O., Rome, who, from an analysis of the growth data obtained from these experiments, was able to demonstrate the general consistency between observations from the field and the laboratory. He concluded that the concurrent rearing of two groups of animals, one fed at a predetermined level and one starved, is a useful method of studying growth parameters and their relation to environmental influences. The details of this analysis will be presented in a separate paper. In the natural population it appears therefore that the gradual decrease in mean size was due to starvation, and the final increase in mean size (14 May–1 August 1956) can be related to maximum summer settlement of barnacles in the presence of greatly depleted numbers of juvenile *Asterias*.

Bull (1934) reared three specimens of *Asterias* from newly settled young to sexual maturity in laboratory tanks at Cullercoats. He recorded the average radius, from the tip of the arm to the centre of the disc, of five arms, and obtained regular growth curves. The three starfish average 42 mm in the June after settling and 114 mm at the time of first spawning. Vevers (1949) followed the growth of individual *Asterias* in the laboratory and Barnes & Powell (1951) recorded a similar and quite rapid growth rate of young *Asterias* on a barnacle-covered raft at Millport. Graphs prepared from some of their figures are included in Fig. 9 for comparison with the present observations. The growth rate shown by the Southward Laying juveniles during the period soon after their settlement, and from June to August 1956, after reduction in numbers by competition had allowed the survivors to grow normally, was very similar to earlier records. Smith (1940) found that where high population densities of *Asterias vulgaris* Verrill occurred in eastern Canada, the size of individuals tended to be smaller. The growth of well fed individuals in our laboratory approached that found by Bull (1934) and Vevers (1949), but these also had been undersized for their age when transferred to laboratory tanks on 1 January 1956. Orton & Fraser (1930) examined a population of *Asterias* on a buoy near Liverpool: Vevers (1949) converted their measurements of diameter to radius sizes, and concluded that the Mersey population had grown considerably more slowly than the individuals in his laboratory experiments.

The use of Nile Blue Sulphate for marking starfish in the field suggested an application for studying growth increments. Five *Asterias* from each group used to study growth in the laboratory were dyed with Nile Blue Sulphate. Their growth during the period 11 February–17 August 1956 was not significantly different from that of undyed starfish, indicating that dyeing did not interfere with their normal growth processes. Eventually white undyed tips were produced on the dyed radii, but the relationship between the undyed and total increments was somewhat variable. The following typical examples showed that up to half the total increment was undyed, suggesting, not very conclusively, that intercalary and terminal growth take place in equal proportions:

Original radius length (mm)	Total increment (mm)	Undyed increment (mm)
19	12	6
19	12	3
19	10	5
18	7	3
18	6	3

It is interesting to note that the stomach pouches of *Solaster*, which had eaten dyed *Asterias*, became coloured blue, giving a useful confirmation of their feeding behaviour.

SUMMARY

Observations on the distribution of adult starfish (*Asterias rubens* L. and *Solaster papposus* (L.)) on an oyster laying in the River Crouch have revealed no marked changes in their numbers or position throughout a period of nearly two years. Slight movement of *Asterias* was recorded, not inshore on to cultivated oyster ground but offshore to more derelict ground infested by slipper limpets (*Crepidula fornicata* Say). This offshore movement may have resulted from the clearing by dredging of large quantities of slipper limpets from the inshore region, necessitating a wider search for food by *Asterias*.

Young *Asterias* settled in 1955 in maximum density inshore on cultivated oyster ground, but some settlement also occurred farther offshore in the area inhabited by *Solaster*. Young *Solaster* settled mainly in mid-river in the region of greatest abundance of adults. Both young and adult *Solaster* have been observed devouring *Asterias*, particularly juveniles, while on a lesser scale *Asterias* may eat young *Solaster*. These facts, coupled with the discrete settling behaviour of *Solaster* are believed to be responsible for the very slight overlapping of the two adult populations.

The very dense population of *Asterias* which settled in 1955 on this oyster ground in the River Crouch has decreased steadily due to the combined effects of prolonged shortage of food, cannibalism and predation by *Solaster*. The dense population of barnacles formerly present on this ground has disappeared and none were seen in 1956. There was no settlement of *Asterias* in that year.

Young *Solaster* showed a steady growth rate, but *Asterias* remained stunted until great reduction of the population allowed normal growth to be resumed. Feeding experiments showed that young *Asterias* grew well on barnacles and young mussels, which they ate readily, but young oysters and *Crepidula* were less acceptable and inadequate for normal growth.

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GROWTH OF OYSTERS (*OSTREA EDULIS* L.)

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Fisheries Experiment Station, Conway

(Text-figs. 1–11)

Although the biology of the oyster *Ostrea edulis* has been well studied, its growth has received little attention. Orton (1928) worked on the populations in the Rivers Fal and Blackwater, but his conclusions have not been supported by Havinga (1928) or Korringa (1955), while America studies on *Crassostrea virginica* have given further evidence of the need for new investigations.

As the Conway breeding experiments necessitate the maintenance of a small oysterage at Tal-y-foel, Anglesey, stocked with oysters of known age and homogeneous origin, a good opportunity was available for studying the growth of marked or isolated oysters. This paper records some observations on the seasonal and annual patterns and on the effect of tidal exposure.

CONDITIONS AND EXPERIMENTAL METHODS

The position of the oysterage is shown in Fig. 1. The beach above mid-tide level is of steeply sloping shingle; but below this level the slope is much more gradual and the beach varies from mud with varying proportions of sand to small shingle bound together by mud and sand. Near Tal-y-foel pier there is a small, exploited, mussel bed; while the oyster grounds are occasionally picked over for periwinkles. The lower part of the shore, where the oysters are kept, is protected from rough weather by a sand bank, Traeth Gwyllt, separating the Tal-y-foel inlet from the main channel. A tidal current of 1–2 knots flows parallel with the shore, associated with a tidal range of about 17 ft. at springs and 10 ft. at neaps.

Salinity samples were taken only at low water springs—the time of lowest salinity—as, when the tide is up, the local drainage water from small streams has less influence. Most of the samples (Fig. 2) were of a salinity of 31‰ or more; lower records were scattered roughly at random throughout the year, and were probably associated with recent heavy rain which, as consecutive samples show, has no lasting effect.

Water temperature was recorded by a thermograph for 17 months. From these records it was found that the mean monthly water temperature could be estimated from the mean monthly air temperature at Valley, 13 miles to the north-west, as published in the Monthly Weather Report of the Meteorological Office (Fig. 3). The regression calculated from this data allows the mean water temperature at the oysterage to be estimated with an error of less than 0·5° C. For consistency, all the mean temperatures quoted in this paper are calculated from the Valley air temperatures using the curve shown in Fig. 3.

Observations on the position of low-water mark on a graduated staff showed that there was a close correlation between the level to which a given tide fell at Tal-y-foel and the level to which the same tide fell in Holyhead Docks (Fig. 4). As a tidal recorder was in continuous use at Holyhead, it was possible to calculate the number of times to which the tide fell to certain levels at Tal-y-foel in a given period. The amount of exposure to the air of

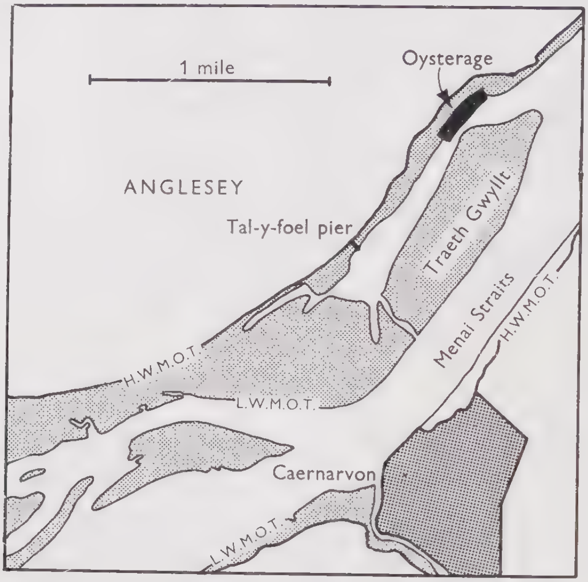
1 mile
Oysterage
ANGLESEY
Tal-y-foel pier
Traeth Gwyllt
Menai Straits
H.W.M.O.T.
H.W.M.O.T.
L.W.M.O.T.
Caernarvon
L.W.M.O.T.

Fig. 1. Map showing the position of the oysterage. The sand banks, exposed at low tide, are lightly stippled.

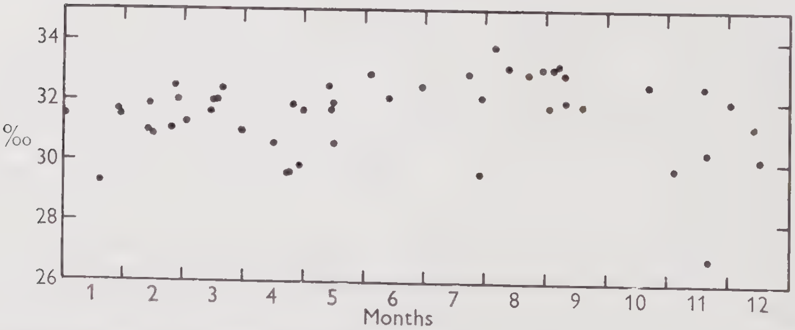

Fig. 2. Salinity of the water at low water of spring tides at the oysterage. The data covers the period December 1951–November 1953.

various levels on the shore by tides of different heights was determined by setting up a continuously recording tide gauge for a period on the pier at Tal-y-foel, the instrument being calibrated in terms of levels at the oysterage. From these values, combined with the data on the frequency to which the tide fell to different levels, a tidal exposure curve for that part of the shore used for the oyster experiments has been calculated (Fig. 5).

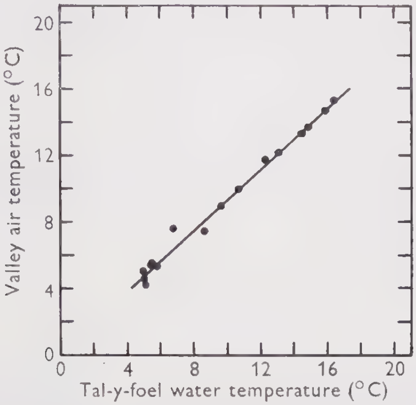

Fig. 3. The relation between the mean monthly air temperature at Valley, Anglesey, and the mean monthly water temperature at Tal-y-foel. The line drawn is the regression giving the best estimate of the water temperature from the air temperature.

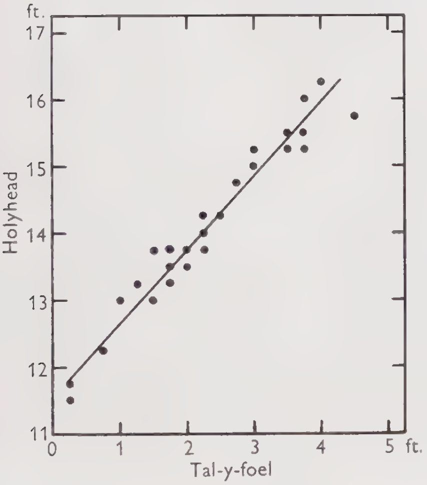

Fig. 4

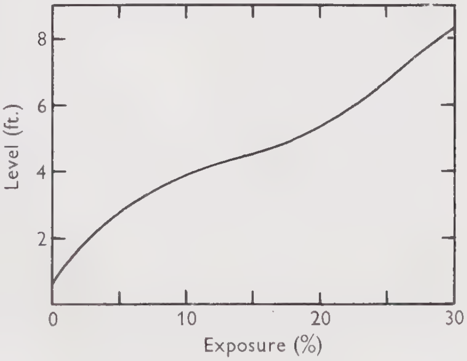

Fig. 5

Fig. 4. The relation between the height of low-water springs at Holyhead and Tal-y-foel. The line drawn is the regression giving the best estimate of the height of tide at Tal-y-foel.

Fig. 5. The percentage of the time for which the lower levels of the shore at Tal-y-foel are exposed.

The general treatment of spat and older oysters in trays was that described and illustrated by Cole (1938). The oysters are first brought to the oysterage when 1–2 months old, attached to earthenware tiles coated with a lime mortar. The tiles are wired together in bundles of ten and stood on racks at low-water mark. When the spat are about 12 months old they are stripped off the tiles and kept in wooden trays with wire tops and bottoms.

The trays in which most of the growth observations were made measure 5 ft. 4 in. by 3 ft. 0 in. by 4 in. deep. The bottoms were of $\frac{1}{2}$ in. woven wire and the top of $\frac{1}{2}$ in. wire-netting. The trays were raised on posts about 18 in. above the mud and were subdivided into three or nine compartments as required.

The diameter used in this work is the mean of the antero-posterior and dorsi-ventral measurements taken to the nearest millimetre below. The weight, when recorded, was measured to the nearest gramme.

SEASONAL AND ANNUAL GROWTH AT LOW-WATER MARK OF SPRING TIDES

Growth at Tal-y-foel has been studied by measuring and weighing at intervals, samples of oysters kept at low-water springs ($< 5\%$ exposure). In some experiments the growth of marked individuals has been followed, and in others the mean increase in size or weight of groups has been recorded. While weight is a more useful parameter of growth than a linear measurement, it is not the easiest to measure in the field. Unfortunately, because of irregularities in shape, it is impossible to estimate reliably an oyster's weight when only the mean diameter has been recorded. A further difficulty is that it is impossible to determine size satisfactorily without killing the specimen; the all-important meat cannot be weighed or measured without opening the shell. The growth of a given individual can only be partially followed since, although the shell can be measured and the whole animal can be weighed, the changes which are recorded may or may not be a reflexion of the health and well-being of the animal within the shell. However, as, in general terms, a big oyster will contain more meat than a small one, conditions which promote a good increase in size will be necessary before there will be a good production of meat. It does not necessarily follow that conditions which will bring about a good increase in external size will necessarily produce good meats, but it is a step in the right direction.

The mean increments in diameter have been recorded for small batches of oysters for various periods in the years 1945, 1952, 1953, and 1956 (Fig. 6). So as to give a satisfactory standard of comparison between periods of unequal length, the recorded increment for each period has been converted to the mean increment in 30 days. In general there was little or no growth between the beginning of November and the end of March, and, in fact, the diameter and weight usually decreased slightly in this period, due to the abrasion of the shell by winter storms. This happened both to oysters laid on the ground and to those in trays, as the following example, which gives the mean weight and mean length of two samples of 200 18-month old oysters, shows:

	In trays		Laid on ground	
	Weight (g)	Length (mm)	Weight (g)	Length (mm)
29. xi. 44	10·8	46·9	10·9	46·6
14. iii. 45	10·3	45·9	10·2	45·3
Decrease	0·5	1·0	0·7	1·3

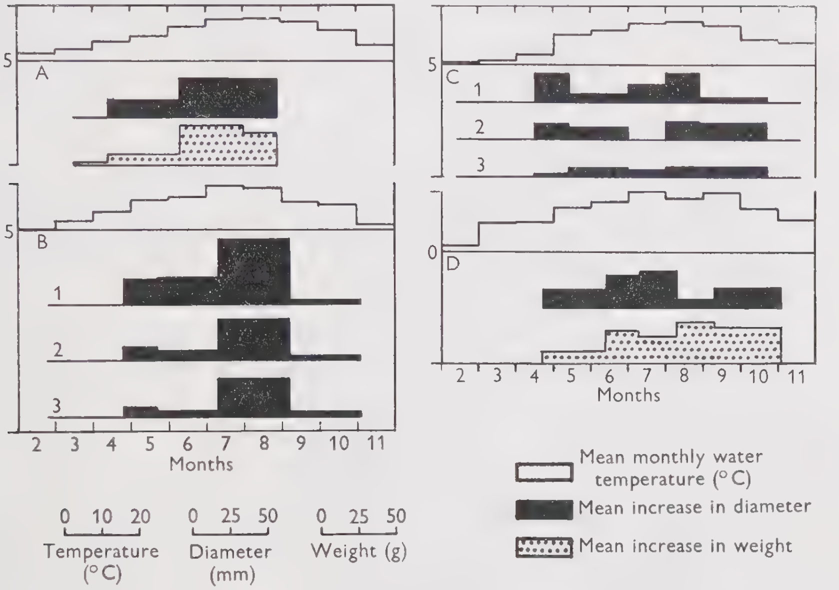

Fig. 6. Seasonal growth at Tal-y-foel. The increment, either mean diameter or mean weight, in the various periods has been calculated as the mean for 30 days. The base-line for all graphs is 0 except for three of the water temperatures where it is 5°. Vertical scales given at bottom left. A. 1945. Growth in mean diameter and weight of 1½-year-old oysters. B. 1952. Growth in mean diameter only of: (1) 1½-year-old oysters; (2) 2½-year-old oysters; (3) 3½-year-old oysters. C. 1953. Growth in mean diameter only of: (1) 2½-year-old oysters; (2) 3½-year-old oysters; (3) 4½-year-old oysters. D. 1956. Growth in mean diameter and weight of 1½-year-old oysters.

In none of three years when observation began in the winter months was growth in mean diameter found in the April samples, although there was a slight increase in weight in 1954. In the samples taken in May growth was general—the mean temperature had risen to about 12° C. Growth continued, the rate varying in different years, until October or early November. In September 1952 the temperature dropped rather sharply to 12·7° C, and little subsequent growth occurred. In 1953 and 1956 when temperatures were higher, with means of 15·8° and 16·0° C in September, and 12·0° and 11·9° C in October respectively, growth continued into October. These results suggest that the beginning and end of a season's growth, both in diameter and total weight, is controlled by temperature. Once the critical limits, about 10°–12° C are passed, other factors, of which food is probably the most important, determines the growth rate. Andreu & Arté (1956) showed that the growth of young oysters in the Ria de Vigo was more closely related to the abundance of small diatoms than to the temperature which was sufficiently high for growth to continue throughout the year. The relationship between water temperature and growth is shown in Fig. 7, where the weight increase between successive measurements, expressed as grammes increase per 30 days, is plotted against the mean temperature for the period. Clearly, considerable variation occurs. The results of August 1956 showed that a substantial increase in weight can take place without a corresponding increase in diameter—an illustration of the inadequacy of linear measurements.

As the observations made in 1953 were on marked oysters, the proportion of the stock which had increased in diameter by various amounts can be calculated; the data for 1952 are less suitable as the samples were not measured sufficiently often. In fig. 8 the proportions of the 3-, 4- and 5-year-old Conway oysters, combined together, which had grown 1 mm or more, 3 mm or more, and 5 mm or more, during various periods in the 1953 growing season are shown. This figure demonstrates that a small proportion put on shell shoot in the period mid-February to mid-April, although the mean diameter of the whole sample did not increase until May (Fig. 6). Apparently the shoot of the growing oysters was counterbalanced by the abrasion of those which were not growing. From mid-April until mid-October between 80 and 90% of the stock put on some shoot during the approximately monthly periods between measurement. Throughout June to October about 60% put on a shoot of 3 mm or more, and about 35% a shoot of 5 mm or more in each period of about a month. The variations from period to period are largely due to differences in the intervals between measurements.

These results differ in some respects from those obtained by Orton (1928) from a study of growth in the Rivers Fal and Blackwater in the years 1926 and 1927. Orton found that growth was confined to temperatures above about 10° C, but believed that, both in the river Fal and the River Blackwater, there were two growing periods, one in the spring and one in the autumn, while in the summer months, June, July and August, there was no increase in diameter. In the Menai Straits, however, the proportion of oysters showing an increase in diameter (Fig. 7) rises during the spring and is maintained at an approximately constant level throughout the summer and drops again in the autumn; there was no period in the summer when growth in diameter did not occur.

Orton suggested that the cessation of growth in the summer was due to the energies of the Fal and Blackwater oysters being taken up by spawning activity, but, according to Korringa (1955), oysters are able to continue growing at a normal rate during the spawning period, provided that feeding conditions are satisfactory. An oyster which was weighed in water daily by Havinga (1928) spawned and brooded its larvae during the period of observation.

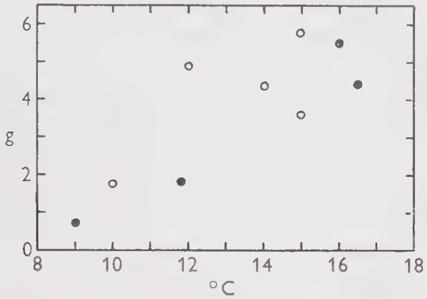

Fig. 7. The relation between mean water temperature and increment in weight in $1\frac{1}{2}$-year-old Conway oysters for various periods in 1945 (solid circles) and 1956 (open circles).

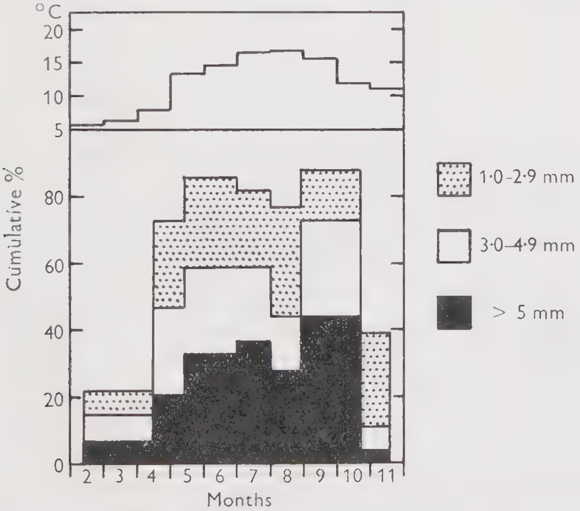

Fig. 8. The percentage of oysters showing shell shoots of varying size in different periods of 1953. The shell shoot has been taken as the maximum increase in either breadth or length.

Growth was retarded for 13 days but stopped only for 4 days. As both the Blackwater and the Fal produce large fat oysters, it is difficult to believe that food is inadequate for simultaneous growth and spawning. Orton, examining at weekly intervals, samples of oysters for the presence or absence of a shoot of new shell growth, decided that few had any shoot in the summer months and concluded that they were not growing. In my experience, the new shoot at the beginning of the year is readily seen, because, during the winter months, the shell becomes uniformly coloured, and the edge abraded, so that when growth starts the new clean white or transparent shell is very readily visible. In the summer, however, two factors disguise the new shoot: the more rapid growth of shell causes the new shoot to harden much more quickly so that less new flexible shell is seen, and, secondly, the increased growth of encrusting organisms, bacterial, algal and animal, colours the shell uniformly. I suggest that if Orton had studied the same oysters throughout the year, considerable growth would have been found during the summer months. The oysters kept as breeding stock in the Conway tanks during the summer months produce a considerable shell shoot despite a high rate of spawning.

TABLE 1. ANNUAL INCREASE IN WEIGHT IN DIFFERENT YEARS OF CONWAY OYSTERS OF DIFFERENT AGES

The oysters were kept in trays at low water mark of spring tides at Tal-y-foel.

Year of observation	Age at beginning of growing season (Years)	Year class	No. of oysters	Initial mean weight (g)	Increment (g)
1955	1½	1953	265	6·43	17·19
1955	2½	1952	272	26·96	17·24
1956	1½	1954	30	16·7	25·3
1956	5½	1950	9	121·42	19·80
1956	6½	1949	8	124·0	8·0
1956	7½	1948	20	129·05	13·65
1957	1½	1955	251	12·6	25·50
1957	2½	1954	342	46·8	22·92
1957	6½	1950	9	141·2	23·5
1957	8½	1948	20	140·3	20·3

From the data obtained on the increase in weight during a season's growth (Table 1), a general growth curve covering the oyster's life span can be suggested. It is convenient to consider such a curve in terms of the actual weight increment laid down each year. In these terms a growth curve is made of three parts: an initial period when the weight increment in a given time increases with increasing size and age of the animal, a middle part where the weight increment is constant, and a third part where, as the curve approaches the asymptote, the weight increment decreases.

To determine accurately the mean increment for a year-class, it is necessary to work with very large samples since any year-class is made up of fast, average and slow growing oysters, a point demonstrated by some data obtained in 1957. At the beginning of the growing season random samples of two year classes, 1955 and 1954, of Conway oysters were collected and the oysters, after being assigned to 4 g weight-groups, were planted in separate tray compartments. At the end of the growing season the mean weight increment per oyster was obtained for each group. Fig. 9, plotted from this data, shows that the larger oysters of a year class have bigger increments than the smaller ones.

Since the oysters were of the same age, differences in initial size must have resulted from more rapid growth which was clearly continued into the experimental period. After several years growth these differences result in a considerable range of weights among any 1-year-group. For example, at the end of 1957, a sample of twenty 8½-year-old oysters, which had been kept continuously in a cage, had individual weights varying from 91 to 235 g.

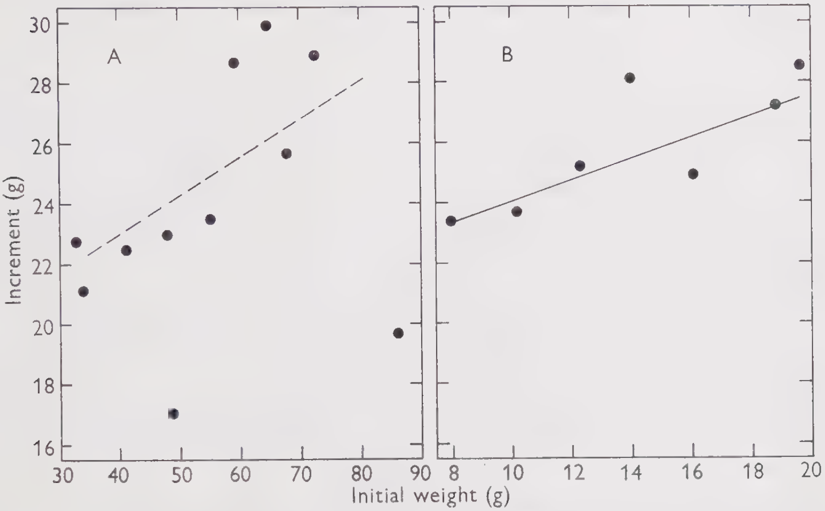

Fig. 9. The weight increment in samples of oysters of different initial weight but the same age during the 1957 growing season. (A) Oysters 3½ years old at the beginning of the growing season. Trend line fitted by eye. (B) Oysters 2½ years old at the beginning of the growing season. The line drawn is the regression giving the best estimate of the increment.

If these differences in growth rate are due, at least in part, to genetic factors, they provide a basis for selective breeding work. The existence of such differences does, however, show that a reliable average growth rate can only be obtained from very large samples.

During the first growing season after settlement, the spat, which weighed around 1–2 g at the beginning, increased in mean weight by 5–15 g, according to the year, so that at the end of this season (when 1½ years old) the mean weight varied from 6 to 16 g. In 1955 and 1957 there was an approximately equal weight increment in each year among oysters in their second and third growing seasons. The increment was larger than that occurring in younger oysters. This conclusion was confirmed (see below) during a study of growth at different levels on the shore. It is clear, therefore, that the change from an increasing size of weight increment with age to a constant increment irrespective of age occurs at about 1½ year old—at the end of the growing season following that in which settlement occurs.

The age at which the size of the increment begins to decline has not been determined because of the scarcity of older oysters of known age. The data for 1956 suggested that a decrease might have started in oysters of $5\frac{1}{2}$–$7\frac{1}{2}$ years old, but this was contradicted by the 1957 data when oysters of $6\frac{1}{2}$ and $8\frac{1}{2}$ years old increased by approximately the same amount as those $1\frac{1}{2}$ and $2\frac{1}{2}$ years old. It may be reasonable to assume that in any 1 year oysters from $1\frac{1}{2}$ years old to at least 5 or 6 years old increase in weight by equal amounts.

EFFECT OF TIDAL EXPOSURE

Some preliminary observations showed that small changes in exposure had a a considerable effect on growth. For example, in April 1947, three plots of oysters newly arrived from Brittany were laid on the ground at low-water mark of neap tides, at low-water mark of ordinary spring tides, and at low-water mark of extreme spring tides, respectively. At the beginning of the experiment the mean wet meat weight of each oyster was 2·62 g. By December, when growth had finished, this had increased at the three levels to 3·6, 4·0 and 5·7 g respectively. A similar experiment was made in 1948 in three stepped trays each 18 in. apart, the lowest being at extreme low water of spring tides. The trays were stocked with a random collection of 18-month-old Conway oysters, which, at the end of the growing season, had mean meat weights of 2·1, 2·6 and 2·8 g at the lowest level. In 1953, three similar stepped trays were prepared, again 18 in. apart, the lowest 'step' being at the level of 1·5% exposure, the middle at 5% and the top tray at 14% exposure. Each tray was stocked with fifteen individually marked Conway oysters, half of them being 2-year-old and the rest 3-year-old; their mean diameters were recorded at intervals. The results, shown in the table below, show little difference between the 1·5% and the 5% exposure level, but at the 14% exposure level growth was much reduced:

	1949 year-group			1950 year-group		
Exposure	1·5%	5%	14%	1·5%	5%	14%
Diameter—March (mm)	70	67	66	62	63	67
Diameter—November (mm)	83	78	73	81	83	74
Increase (mm)	13	11	7	19	20	7

The data, when plotted, suggest that growth would have stopped at about 25–30% exposure. The amounts of growth made during different parts of the season varied similarly at all levels.

In 1955 a more detailed investigation was made. Six identical trays were erected individually 1 ft. above the level of the mud at positions on the shore ranging from 1 to 23% exposure. A further two trays were erected on much longer posts in order to compare the growth of oysters at the same tidal level but at different distances above the mud. Each tray was stocked with 100 1-year-old and 100 2-year-old Conway oysters; the two age-groups were kept separate. The diameters and weights of all oysters were recorded in April and October.

The results showed (Fig. 10) that growth was sensitively related to the percentage exposure to the air. Although the point of no growth was not quite reached, extrapolation suggests that it lay at the tidal level of about 30% exposure, which agrees with the estimate from the 1953 experiment. If it is a simple relationship between growth and exposure, the data suggests that about three-quarters of the feeding activity is devoted to basal metabolism and the elaboration of reproductive products, while about one quarter is devoted to growth.

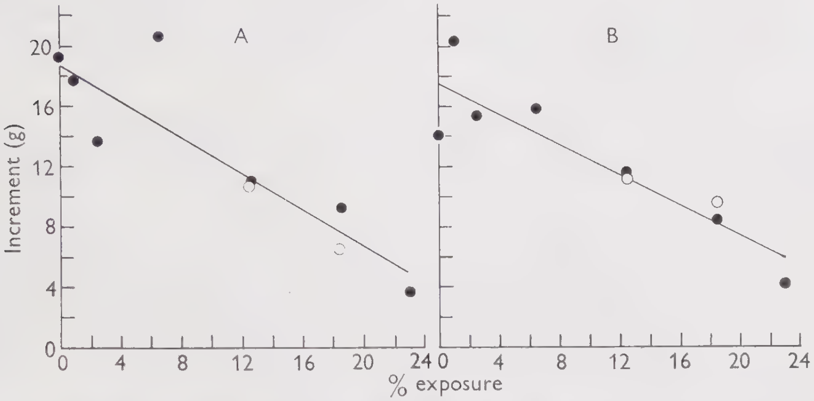

Fig. 10. The weight increment in 1955 of samples of 100 oysters exposed at different levels on the shore. Solid circles—trays standing about 18 in. above the mud. Open circles—trays standing about 4 ft. above the mud. (A) 2½-year-old oysters. (B) 1½-year-old oysters.

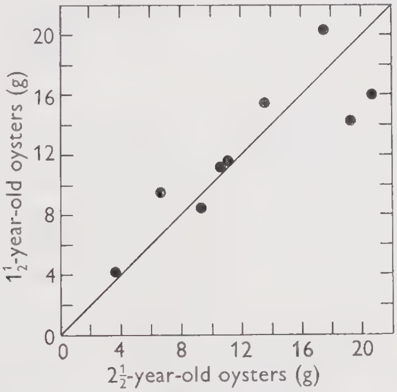

Fig. 11. A comparison of the weight increase of oysters of two age-groups, both exposed at different levels on the shore. The line drawn is that of equal increment for each age-group.

The ratio of the shell weight to the meat weight was found to be approximately the same in samples examined from the different trays. During the experimental period of 6 months no observable systematic tendency towards the development of relatively heavier shells was discernable at any level.

It is noteworthy that at each level the mean increase in weight was the same for both the 2- and 3-year-old oysters (Fig. 11). Apparently the conclusion reached previously in this paper, that in any year the actual increase in weight is constant for oysters of at least two year-groups, holds when the physical conditions are altered.

I am indebted to Captain F. Arden, District Marine Manager of British Railways, for supplying me with details of the tidal levels at Holyhead.

SUMMARY

A description is given of the Tal-y-foel oysterage. Records show that the mean monthly water temprature can be accurately estimated from the mean monthly air temperature recorded at Valley, Anglesey. A tidal exposure curve has been calculated for the shore below mid-tide level. Salinity samples taken at low water of spring tides rarely fell below 30‰.

The growth of oysters, determined by length measurements and by weighing, was confined to the period April to October inclusive, that is when the water temperature was above 10–12° C.

The rate of growth, and the proportion growing, increased during the spring, was approximately constant during the summer, and declined during the autumn. No evidence was found for the view that growth is confined to the spring and autumn.

In any given year the mean increase in weight is the same for 1½-year-old and 2½-year-old oysters. In younger oysters the size of the increment increases with increasing age. The size of the increment does not begin to decline until oysters are at least 5–6 years old and probably older.

Growth was strongly affected by tidal exposure. The amount of growth decreases steadily until at the level of about 30% exposure no growth occurs.

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OBSERVATIONS ON THE INCREASE IN SIZE AT MOULTING IN THE LOBSTER (*HOMARUS VULGARIS* M.-EDW.)

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(Text-fig. 1)

A knowledge of growth rates is a pre-requisite in estimating the effect of fishing upon the available stocks. In Crustacea, where there is no known means of establishing accurately the age of the individual, the importance of measuring the growth rate is increased whilst its determination is made more difficult. In *Homarus vulgaris* some experiments were undertaken by Dannevig (1936), and Wilder (1953) gives considerable data for the American lobster. Results suggest that the growth increment is not uniform in all latitudes. Experiments to augment the limited data available for *H. vulgaris* and to establish the increase in size at moulting in local lobster stocks were therefore undertaken by the Marine Laboratory of the Scottish Home Department at Aberdeen. A statement of some preliminary results was given in *Report on the Fisheries of Scotland* (Lucas, 1957, p. 58).

MATERIAL AND METHODS

The present investigations were carried out off the south-east Scottish coast. The area is characterized by an intensive creel fishery and heavy fishing mortality (Thomas, 1955*a*). A high level of returns could therefore be expected. Off the Berwickshire coast the onset of the main moulting season for lobsters occurs in about the middle of May. During the period 7–13 May, 1956, a total of 1012 lobsters were purchased from local fishermen, and, having been marked to give an indication of their size, were released on to the fishing grounds. Punch markings on the telson and tail fan were coded so as to indicate the existing minimum carapace length of the lobster, in 1 mm groups, measured from the back of the eye socket to the posterior end of the carapace.

Over the period September 1956 to April 1957 altogether 111 marked lobsters were returned which had moulted in the intervening period. The carapace length of the recovered lobsters together with a note of the initial length, as shown by the code markings, and the sex were recorded.

The present series of experiments has been limited to the narrow size range around the minimum legal landing length, because this is the most important class from the standpoint of population dynamics. Restriction of the size range also renders the experiment more profitable since the smaller lobsters are more likely to have moulted before recapture. It also facilitates a system of coding into small groups, in this instance 1 mm.

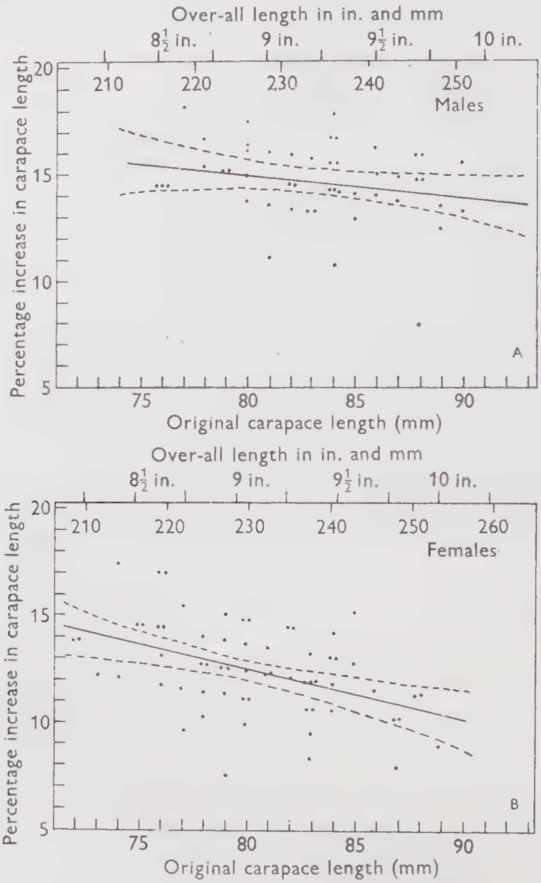

Fig. 1. (A) The percentage increase in the carapace length of male lobsters at moulting. (B) The percentage increase in the carapace length of female lobsters at moulting. The 95 % confidence limits are shown by broken lines.

The timing of the liberations, immediately prior to the main moulting season, was also chosen so that the greatest numbers of marked lobsters would have moulted before being recaptured. However, the possibility of a different increase in size at moult occurring in lobsters moulting other than at the peak season cannot be excluded.

RESULTS

The percentage increase in carapace length at moulting based on a sample of 49 male lobsters is shown in Fig. 1 *a*. At carapace length 80·9 mm, equivalent to an over-all length of 9 in. (230 mm) which is the minimum legal landing size, it is about 15%. Smaller lobsters have a somewhat higher increment whilst larger individuals gain less. In terms of weight the increase of a 9-inch lobster at moulting is roughly 55%.

The comparable moult increments for a sample of 62 female lobsters is shown in Fig. 1 *b*. The percentage increase at the moult is materially less than in the male lobsters, whilst the decrease in the moult increment with increasing size is more marked. At carapace length 79·6 mm, equivalent to an over-all length of 9 in., the growth at moulting is about 12·75%. In terms of weight the minimum legal-sized female lobster increases roughly 41%.

Statistical analysis shows that the moult increments of male and female lobsters, of legal landing size, differ significantly ($P < 0{\cdot}001$). The levels of moult increments are broadly comparable with those found by Dannevig (1936) for *H. vulgaris* and by Wilder (1953) for the American lobster, although in the latter a lower increment in females does not seem to have been observed. This, however, may well be because most of Wilder's observations were made on smaller lobsters. The difference in growth between the sexes is probably associated with maturity which would explain the more rapid fall-off in moult increment with increasing size of the females as opposed to that in the males. In the present experiments no specimens were returned showing abnormally high growth rates or physical characteristics suggesting that they had moulted twice during the period of liberty.

DISCUSSION

Simpson (1958) found that in North Wales lobsters had a moult increment which is lower than that found off Berwickshire. A striking fact is that out of only 41 returns in the North Wales experiments, 3 lobsters had moulted twice within a period of about 7 months. None of the 111 returns from the Berwickshire coast had moulted twice, although the average size of lobsters marked was lower. It would seem reasonable that the higher average moulting frequency should be accompanied by a lower increase in size at each moult.

The present experiments give a measure of the increase in size at the moult. From the population standpoint the important measure is the *annual* growth rate. It is difficult to envisage any marking experiments which would alone give a true measure of the annual growth rate. Nevertheless, this can be derived from the results of marking experiments in conjunction with data on the frequency of moulting. Unfortunately, knowledge of the moulting frequency in the various length groups is not precise. In certain areas a proportion of 9-in. lobsters cast twice annually (Thomas, 1958). The incidence of such moulting probably varies with sea temperature. Off Berwickshire it would seem probable that few 9-in. lobsters moult twice annually. Male lobsters of between 10 and 11 in. moult only once a year. Female lobsters of this size fall into two classes—those which do not mature but moult annually; those which mature, spawn and have an inter-moult period which is normally 2 years. The normal moulting frequency of the female, however, may be upset at about maturity so that the last inter-moult period prior to maturity is reduced or the interval between moulting and spawning is less than normal. Such irregularities seem in general to be associated with an abnormally small moult increment in the second moult or a fecundity below average. In view of the inadequate knowledge of the incidence of such occurrences, it is preferable to concentrate primarily on the male lobster in using growth rates for calculation of mortality rates.

It has been noted that the female lobster approaching maturity has a lower moult increment than the male, although in smaller lobsters the rates may be similar. This will have some bearing on the sex ratio of the stock. The lower growth rate will increase the proportion of females to males in the sizes immediately above the level at which the growth rates diverge. There will also be an accompanying decrease in the proportion of females to males amongst larger lobsters. The effect is similar to and precedes that arising on account of the difference in over-all growth rates which is associated with the increased inter-moult period in the spawning female (Thomas, 1955*b*).

SUMMARY

The percentage increase in carapace length at moulting of male lobsters off the south-east Scottish coast is about 15 % and for females 12·75 %.

The rate of moulting increase in carapace length falls off with increasing size in both male and female lobsters of above 8 in. over-all length, but the decrease is more pronounced in the female.

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THE OCCURRENCE OF GALLIUM IN MARINE ORGANISMS

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Little is known about the occurrence of gallium in the biosphere. The first recorded instance of its detection in a living organism is due to Cornec (1919), who detected the element in *Laminaria* sp. Vinogradov (1935) suspected its presence in an undetermined gorgonian, but his statement that Zvinden had detected it in human tissue appears to be an erroneous citation (Hutchinson, 1943). Bertrand (1941) detected the element in oysters, and, after chemical concentration, in human urine.

Noddack & Noddack (1940) have determined gallium spectrographically in nine marine organisms. They found concentrations (expressed as parts per million on the dry weight) ranging from 0·1 p.p.m. in *Squalus acanthias* to 0·7 p.p.m. in the body walls of *Asterias rubens* and *Bryssopsis lyrifera*. The average amount recorded (0·4 p.p.m.) corresponds to a concentration factor over sea water of 16,000 (assuming the gallium concentration of 0·025 μg/l. in sea water, found by Burton, Culkin & Riley, 1958). The element has also been detected in *Lycopodium flabelliforme* (0·1 p.p.m.) and *Mitchella repens* (0·1–0·2 p.p.m.) (Hutchinson & Wollack, 1943); in ambergris (Ishiguro, Koga & Matsuo, 1952); in the outer layers of some seeds and fruits, and in several species of beetles (Bergman, Borovik & Borovik-Romanova, 1943; Borovik & Borovik-Romanova, 1944, 1949). From these investigations the concentration of gallium in living organisms appears to be about $n \times 10^{-5}\%$.

The biological role of gallium has been the subject of several investigations. Steinberg (1938, 1939*a*, *b*; 1941) has found that trace amounts of gallium are necessary for the growth of *Aspergillus niger* and *Lemna minor* in sterile medium. More recent investigations by Bertrand (1954) are, however, at variance with these conclusions. G. A. Riley (1943) has reported that in nutrient deficient culture the growth of the marine diatom, *Nitzschia closterium**, is stimulated by gallium, but that this effect disappeared when nitrate and phosphate were present. No definite conclusions can be drawn from this study since no control experiments were made omitting gallium alone. Hewitt & Bolle-Jones (1952) have found no evidence that gallium is an essential micronutrient for the growth of tomato, lettuce or sugar beet. Bardet, Levaditi, Tchakirian & Vaisman (1931) have studied the distribution of gallium in the organs of rabbits given gallium tartrate in their diet.

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* i.e., *Phaeodactylum tricornutum*.

In a review article on the biogeochemistry of aluminium and some other elements of Groups III*a* and III*b* of the Periodic Table, Hutchinson (1943) has pointed out that in many of its properties (gallous and gallic salts, complex cyanides) gallium shows a greater resemblance to iron than to aluminium. He concluded that it might play a part in metabolism similar to iron, but on a restricted scale. Gallium deficiencies are only likely to occur rarely, since few silicate rocks or soils contain less than 5 p.p.m. of the element.

Specific spectrophotometric methods for the determination of gallium in silicates and other minerals have been developed recently (Onishi & Sandell, 1955; Culkin & Riley, 1958). These procedures have sensitivities sufficiently high to allow the estimation of less than 0·1 p.p.m. of gallium using samples weighing less than 5 g. Using a modification of these methods, a study has been made of the distribution of the element in several marine organisms, derived mainly from the Irish Sea and the shores of the Isle of Man. In several of the samples the concentrations of iron, copper and aluminium have also been determined for comparison.

METHODS

Determination of gallium in marine organisms and shells

Reagents

(1) 7·5 N nitric acid.

(2) Perchloric acid, 60% (w/w).

(3) Titanous chloride solution, 15% (w/v).

(4) Di-*iso*-propyl ether. Freshly distilled from sodium hydroxide.

(5) Hydrochloric acid (6·5 N) containing 1% titanous chloride. Concentrated hydrochloric acid (S.G. 1·16, 325 ml.) was mixed with 33 ml. of 15% titanous chloride solution and diluted to 500 ml.

(6) Rhodamine B solution. A solution was prepared containing 0·5 g of Rhodamine B in 100 ml. of water. The solution was filtered before use.

(7) Carbon tetrachloride-chlorobenzene solvent. Carbon tetrachloride (125 ml.) was diluted to 500 ml. with chlorobenzene.

(8) Standard gallium solution (5 μg/ml.) was prepared by dissolving 0·0438 g of caesium gallium sulphate in water and diluting to 1 l.

Solution of samples of shells (carbonates)

The weighed sample (5–10 g) was placed in a 250 ml. conical flask and 50 ml. of 7·5 N nitric acid was gradually added. After effervescence had ceased, the flask was heated gently on a hot-plate for 30 min, and the nitric acid was then evaporated. The residue was twice evaporated to dryness with 5–10 ml. of hydrochloric acid, to remove the nitrate, and dissolved in 50 ml. of 6·5 N hydrochloric acid containing 1% of titanous chloride.

Solution of samples of marine organisms

The weighed sample (up to 5 g, dried at 110° C) was placed in a 250 ml. conical flask, and 25 ml. of 7·5 N nitric acid was added. The flask was closed with a small funnel and its contents were allowed to digest in the cold until all foaming had ceased. The nitric acid was then removed by cautious evaporation on the hot plate. Repeated evaporations with 15 ml. portions of concentrated nitric acid were then carried out until all carbonaceous material was removed. The white or pale yellow residue was treated with 2 ml. of 60% (w/w) perchloric acid and fumed to dryness. It was fumed to dryness again after addition of 2 ml. of concentrated sulphuric acid, and dissolved in 50 ml. of 6·5 N hydrochloric acid containing 1% of titanous chloride.

Extraction of gallium

The solution of the sample was transferred to a 250 ml. separating funnel, and, if it was not violet in colour, an excess of 15% titanous chloride was added. Two extractions with 30 ml. portions of di-*iso*-propyl ether were carried out and the combined extracts were evaporated in a beaker on the water bath.

Photometric determination of gallium

The residue in the beaker was dissolved by warming to 70–80° with 5 ml. of 6·5 N hydrochloric acid containing 1% titanous chloride. The cold solution was transferred to a 50 ml. separating funnel containing 8 ml. of chlorobenzene-carbon tetrachloride solvent. The beaker was rinsed with a further 1 ml. of 6·5 N hydrochloric acid and the washings added to the funnel. Rhodamine B solution (0·5 ml.) was added and the separating funnel was shaken mechanically for 10 min. After the two phases had separated, the organic phase was run through a plug of glass wool into a 10 ml. calibrated flask containing 1 ml. of ethyl alcohol. The aqueous phase was washed with a further 1 ml. of the solvent and the washings added to the calibrated flask, which was then filled to the mark with the solvent. The optical density of the solution was measured at 562 mμ in a 1 cm cell. The reagent blank was determined in the same manner but omitting the sample. The method was calibrated using 1, 2, 4, 6, 8 and 10 μg of gallium. It was found that 5 μg of gallium gave an optical density of 0·565 at 562 mμ in a 1 cm cell. Recoveries of gallium added to biological samples averaged 98%.

Determination of sulphated ash, iron, aluminium and copper

The dried (110° C) sample (1 g) was weighed into a 25 ml. platinum crucible and allowed to stand for a few hours with *ca.* 5 ml. of concentrated nitric acid and 2 ml. of perchloric acid. The crucible was then carefully heated on a water bath until the nitric acid had evaporated. The evaporation with concentrated nitric acid was repeated until all organic matter had been destroyed. The perchloric acid was then fumed off under an infra-red heater, 2 ml. of concentrated sulphuric acid was added and the heating was continued until no further fumes were evolved. The contents of the crucible were treated with 2 g of powdered ammonium carbonate and heated at 450° C. in a muffle furnace for 30 min. The crucible was allowed to cool in the desiccator and weighed.

The sulphated ash was treated with 2 ml. of concentrated hydrochloric acid and 15 ml. of water and heated on the water bath; the resultant solution was filtered into a 250 ml. graduated flask. The residue (if any) in the crucible was washed well with hot water and the washings added to the graduated flask. The solution was diluted to volume and used for the spectrophotometric estimation of iron and aluminium as described by Riley (1958), and for copper as described by Riley and Sinhaseni (1958).

OBSERVATIONS

The composition of marine organisms

Gallium, aluminium, iron and copper have been determined in a number of marine plants and animals which were obtained mainly from the Irish Sea off Port Erin and Port St Mary. Unless otherwise stated the analyses were carried out on the whole organism, which had been washed thoroughly with distilled water and dried at 110° C. In addition, the distribution of gallium and copper in certain organs of *Buccinum undatum* L., *Chlamys opercularis* (L.), *Porania pulvillus* (Müll.) and *Pecten maximus* (L.) has been studied. Specimens of *Pecten maximus* were analysed directly after removal from their habitat and also after being allowed to purge themselves in clean sea water for 7 days. The results of the analyses are shown in Tables 1–6.

TABLE 1. OCCURRENCE OF GALLIUM, ALUMINIUM, IRON AND COPPER IN MARINE ALGAE

Organism	Ash (%)	Ga (μg/g)	Cu (μg/g)	Al (μg/g)	Fe (μg/g)	Ga/Al × 10⁴	Ga/Fe × 10⁴	Ga in ash (μg/g)
CHLOROPHYCEAE								
Codiaceae								
Codium sp.	56·2	0·16	85·5	1336	470	1·2	3·4	0·3
Halimeda gracilis	123	0·05	22·5	143	342	3·5	1·5	0·04
PHAEOPHYCEAE								
Fucaceae								
Ascophyllum nodosum	22·1	0·06	6·2	83·4	61·4	7·2	9·8	0·3
Fucus serratus	30·6	0·03	5·6	101	153·7	3·0	2·0	0·1
F. spiralis	9·2	0·01	15·0	34·4	33·9	2·9	3·0	0·1
F. vesiculosus	24·8	0·03	18·0	51·4	36·4	5·8	8·2	0·1
Pelvetia canaliculata	24·2	0·10	17·9	372	120·8	2·7	8·2	0·4
Laminariaceae								
Laminaria digitata	37·5	0·07	6·2	101	40·9	7·0	17	0·2
RHODOPHYCEAE								
Corallinaceae								
Corallina officinalis	114	0·56	44·7	4420	4680	1·3	1·2	0·5
Lithothamnion sp.	113	0·53	15·7	3570	3420	1·5	1·5	0·5
Lithophyllum sp.	121	0·23	18·1	243	374	1·0	0·6	0·2
Gigartinaceae								
Gigartina stellata	16·7	0·02	32·8	94·8	102	2·1	2·0	0·1
Rhodomelaceae								
Polysiphonia lanosa	24·9	0·19	41	478	493	4·0	2·6	0·8
Rhodymeniaceae								
Rhodymenia palmata	117	0·04	24·4	175	252	2·3	1·6	0·03

The range of gallium concentrations found for the various whole organisms was 0·01–0·96 p.p.m., which is in good agreement with the data for nine marine animals, published by Noddack & Noddack (1940) (0·1–0·7 p.p.m.). Hutchinson (1943) has deduced from the Noddacks' results that gallium is concentrated biologically from sea water to a greater extent than aluminium. This conclusion must be reconsidered in the light of more recent determinations of both aluminium and gallium in sea water. Greenhalgh & Riley (unpublished work) have found aluminium concentrations of *ca.* 10 μg/l. in the

TABLE 2. OCCURRENCE OF GALLIUM, ALUMINIUM, IRON AND COPPER IN MARINE ANIMALS

Organism	Ash (%)	Ga (μg/g)	Cu (μg/g)	Al (μg/g)	Fe (μg/g)	Ga/Al $\times 10^4$	Ga/Fe $\times 10^4$	Ga in ash (μg/g)
PROTOZOA								
Foraminifera								
Ramulina sp.	116	0·18	111	973	1148	1·9	1·6	0·2
PORIFERA								
Halichondria panicea	88·7	0·93	59·5	3700	4040	2·5	2·3	1·1
COELENTERATA								
Alcyonium digitatum	69·0	0·05	61·5	435	438	1·2	1·1	0·1
CRUSTACEA								
Cirripedia								
Balanus balanoides	120	0·07	28·7	240	146	2·9	4·8	0·05
Decapoda								
Cancer pagurus	79·0	0·03	35·0	166	157	1·8	1·9	0·04
Corystes cassivelaunus	71·6	0·36	—	1041	1045	3·5	3·4	0·5
MOLLUSCA (Shells only)								
Lamellibranchia								
Mytilus edulis	—	0·010	2·1	38	41	2·6	2·4	0·01
Pecten maximus	—	0·008	6·0	81	118	1·0	0·7	0·01
Chlamys opercularis	—	0·069	3·8	174	239	4·0	2·8	0·07
Gastropoda								
Buccinum undatum	—	0·036	1·6	76	85	4·7	4·2	0·04
Littorina littorea	—	0·011	5·2	32	45	3·4	2·4	0·01
Gibbula umbilicalis	—	0·014	7·9	48	51	2·9	2·7	0·01
MOLLUSCA (Soft parts)								
Lamellibranchia								
Mytilus edulis	15·8	0·16	53·7	465	325	3·4	4·9	1·0
Pecten maximus				See Table VI.				
Chlamys opercularis	15·7	0·05	37·1	186	1093	2·7	0·5	0·3
Gastropoda								
Buccinum undatum	8·8	0·007	53·5	198	86	0·35	0·8	0·08
Littorina littorea	12·9	0·06	50·2	198	171	3·0	3·5	0·5
L. littoralis	9·0	0·05	102	238	229	2·1	2·2	0·5
Patella vulgata	17·7	0·04	—	346	1415	1·2	0·3	0·2
ECHINODERMATA								
Asteroidea								
Asterias rubens	49·2	0·10	34·7	159	332	6·3	3·0	0·2
Henricia sanguinolenta	70·5	0·05	20·4	652	925	0·8	0·5	0·1
Luidia ciliaris	42·6	0·03	59·0	152	149	2·0	2·0	0·1
Marthasterias glacialis	64·5	0·02	37·7	142	186	1·4	1·1	0·03
Porania pulvillus	65·2	0·11	90·0	365	457	3·0	2·4	0·2
Stichastrella rosea	88·5	0·10	35·0	263	271	3·8	3·7	0·1
Echinoidea								
Echinus esculentus	111	0·03	76·0	597	597	5·0	5·0	0·03
Spatangus purpureus								
Whole organism − gut	122	0·35	36·9	1830	1700	1·9	2·1	0·3
Gut + contents	124	3·7	31·6	16,480	14,800	2·2	2·5	3·0
Ophiuroidea								
Ophiocomina nigra	105	0·09	29·5	894	1600	1·0	0·6	0·1

TABLE 3. DISTRIBUTION OF GALLIUM, ALUMINIUM, IRON AND COPPER IN *CHLAMYS OPERCULARIS*

Organ	Ash (%)	Ga (μg/g)	Cu (μg/g)	Al (μg/g)	Fe (μg/g)	Ga/Al $\times 10^4$	Ga/Fe $\times 10^4$	Ga in ash (μg/g)
Shell	—	0·07	3·8	174	239	4·0	2·9	0·07
Muscle	6·5	0·06	47·9	773	52·8	0·8	1·1	1·0
Gonad	10·4	0·06	—	—	—	—	—	0·6
Mantle and viscera	21·6	0·55	1150	3420	2720	1·6	2·0	2·5

TABLE 4. DISTRIBUTION OF GALLIUM AND COPPER IN *BUCCINUM UNDATUM*

	Mantle	Operculum	Digestive gland	Digestive tract	Mucous gland	Foot	Female ducts	Male ducts
Sulphated ash (%)	5·0	3·7	4·1	5·7	20·4	4·1	3·3	5·9
Ga (μg/g)	<0·01	<0·01	0·014	<0·01	<0·01	<0·01	<0·01	—
Cu (μg/g)	33·4	25·7	80·4	29·2	26·9	2·5	13·7	28·8
Ga in ash (μg/g)	<0·2	<0·3	0·28	<0·2	<0·05	<0·25	<0·3	—

	Renal organ	Female gonads	Ctenidium	Heart
Sulphated ash (%)	10·0	2·1	10·9	5·4
Ga (μg/g)	—	—	—	—
Cu (μg/g)	30·0	27·7	50·3	87·0
Ga in ash (μg/g)	—	—	—	—

TABLE 5. DISTRIBUTION OF GALLIUM IN *PORANIA PULVILLUS*

	Skin	Digestive gland	Stomach + oral region	Skin from aboral region
Sulphated ash (%)	—	11·3	95	60·4
Ga (μg/g)	0·5	0·02	0·20	0·09
Ga in ash (μg/g)	—	0·18	0·21	0·15

TABLE 6. DISTRIBUTION OF GALLIUM, ALUMINIUM, IRON AND COPPER IN *PECTEN MAXIMUS*

Unpurged	Full gonad	Spent gonad	Striped muscle	Unstriped muscle	Mantle	Digestive gland	Gonad + gut	Mantle + gut + digestive gland
Sulphated ash (%)	10·7	3·2	6·0	4·7	13·3	7·2	12·6	13·9
Ga (μg/g)	<0·01	0·009	<0·01	<0·01	<0·01	0·79	0·29	0·30
Al (μg/g)	—	—	86·6	—	161	454	833	842
Fe μg/g)	—	—	167	—	189	841	1552	766
Cu (μg/g)	—	25·2	4·0	—	—	153	—	50·8
Ga in ash (μg/g)	<0·1	0·28	<0·2	<0·2	<0·1	11·0	2·3	2·2

Purged	Gills	Male gonad	Female gonad	Striped muscle	Gut + digestive gland	Mantle
Sulphated ash (%)	25·0	3·0	10·2	12·7	10·9	9·4
Ga (μg/g)	0·14	0·034	<0·01	<0·01	0·011	0·01
Cu (μg/g)	6·3	11·3	26·6	7·5	70·9	10·2
Ga in ash (μg/g)	0·56	1·1	<0·1	<0·1	1·0	<0·1

Irish Sea and English Channel; Monaghan, Simons & Taggert (1953) have detected similar amounts in the Atlantic Ocean and in the waters of the Gulf of Mexico. These amounts are approximately two orders of magnitude less than earlier work had indicated (cf. Richards, 1957). Assuming an aluminium content of 10 μg/l. and a gallium content of 0·025 μg/l. (Burton, Culkin & Riley, 1958), the Ga:Al ratio in sea water is 25×10^{-4}. The average Ga:Al ratio in the lithosphere is *ca.* 2×10^{-4}, which shows that sea water is enriched approximately tenfold in gallium relative to aluminium compared with the lithosphere.

The average value of the Ga:Al ratio for the marine organisms examined in this paper was $2\text{–}3 \times 10^{-4}$, which is very similar to the average ratio for the lithosphere (*ca.* 2×10^{-4}). Since the majority of the specimens examined were either bottom-living or from shallow water it seems very probable that most of their aluminium and gallium was derived from the bottom muds rather than from the sea water itself. The actual range found for the Ga:Al ratio is small ($1\text{–}7 \times 10^{-4}$). This suggests that there is no significant biological separation of gallium from aluminium in the specimens examined, if allowance is made for variations in the Ga and Al contents of the muds in the various localities in which they lived.

The iron content of the organisms is generally similar to their aluminium content, but the Ga:Fe ratio shows rather greater variations than the Ga:Al ratio. The variation in the iron concentration is probably related to its role as an essential trace element in biological systems.

There is apparently no relationship between species and their contents of gallium, iron, aluminium or copper. The low concentrations of these elements found in the calcareous shells of molluscs suggests that the mechanism by which the calcium of sea water is converted into calcium carbonate is quite selective for calcium. The gallium contents of the shells are similar to those of carbonate rocks (Culkin & Riley, unpublished). All four elements are concentrated in the digestive glands and viscera of the molluscs, but it seems probable that the gallium, aluminium and iron contents of these organisms are associated with ingested detritus. This is confirmed by the low concentrations of these elements present in the gut and digestive glands of *Pecten maximus* specimens, which had been allowed to purge themselves in filtered sea water for 7 days. The copper contents of the purged and unpurged specimens were similar, and it is probable that the copper in them is mainly adsorbed on to the mucous surfaces.

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SUMMARY

A spectrophotometric method has been developed for the determination of submicrogram amounts of gallium in biological materials. Using this procedure a study has been made of the occurrence of gallium in a number of marine plants and animals (dried at 110° C). Data are also presented for the concentrations of iron, aluminium and copper in the same samples.

The gallium content of the samples ranged from *ca.* 0·01 to 0·96 p.p.m. The Ga:Al ratio varied from $1–7 \times 10^{-4}$ (average $2–3 \times 10^{-4}$), which compares well with the average Ga:Al ratio of the lithosphere, and contrasts with the ratio of 25×10^{-4} for sea water. It seems likely that the organisms examined derived their gallium, aluminium and iron from the bottom muds rather than from the sea water. There is no evidence of selective uptake of gallium in preference to aluminium. Iron and aluminium were generally present in roughly equal amounts.

The distribution of gallium in the various organs of *Pecten maximus*, *Buccinum undatum*, *Chlamys opercularis* and *Porania pulvillus*, has been investigated. Most of the tissues of these molluscs contain less than 0·07 p.p.m. of gallium, but the viscera and digestive organs contain higher concentrations, presumably contained in ingested inorganic material. The average gallium content of carbonate shells is 0·02 p.p.m.

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THE POLYCHAETE *MAGELONA ALLENI* N.SP. AND A RE-ASSESSMENT OF *MAGELONA CINCTA* EHLERS

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(Text-figs. 1–3)

Three species of the polychaete genus *Magelona* are known from the neighbourhood of Plymouth. One is the widely distributed *M. papillicornis* F. Müller, described first from the coast of Brazil (Müller, 1858) and subsequently frequently recorded for northern European coasts and the Mediterranean. Near Plymouth this species occurs in clean sand in the lower tidal region and offshore, as in Whitsand Bay. Of the other two species, one from clean sand near low water at Mill Bay, Salcombe, awaits description. The third has been known for many years but until now has not been recognized as an undescribed species. It was formerly abundant in the Rame Mud (a deposit of black sandy mud); thus Mare (1942, p. 542, as *M. cincta* Ehlers—a provisional identification by me) on 12 July 1939 found 120 per m^2, associated with large numbers of other polychaetes, lamellibranchs, etc. The locality was close to Ford's station No. 93 (Ford, 1923, chart facing p. 167, and p. 218 where the position is given as Rame Head, E. ½ N. Tregantle, N. ½ E.). At this station on 20 February 1923 Ford had four specimens (recorded as *M. papillicornis*) from $\frac{1}{5}$ m^2. On 11 August 1922 at station No. 53 close by (Rame Head, E., 1½ miles) he obtained thirteen specimens of the same species from $\frac{3}{10}$ m^2, the substratum being black mud. Ford records *papillicornis* from other offshore mud or muddy-sand stations over a fairly wide area inside and outside Plymouth Sound, but in less abundance. It is certain that some of these records, especially those detailed above, are of the species taken by Mare and provisionally identified as *cincta*. In 1939 Ford showed me his collections from the Rame Mud and the *Magelona* worms in them were banded with red pigment as is *cincta*, a character which serves to separate them from *papillicornis* which has no such pigmentation. Much more recently Holme (1953) has had this red-banded species in smaller numbers at several stations some miles south of the Sound and to the eastward of the Eddystone, in sandy and muddy sand grounds, sometimes with *papillicornis* whose range the red-banded species overlaps. During the first part of 1958 many attempts have been made to find a locality where these worms can again be obtained in large numbers. Unfortunately the Rame Mud locality is a dumping ground for rubbish and seems to have changed in character of recent years. Search in the area has yielded only two short anterior portions one of which, narcotized in 7% magnesium chloride in tap water and fixed in hot Bouin's Fluid before preservation in alcohol, forms the basis of the drawings in Fig. 1 C–E. This specimen shows very little contraction from life. The remainder of the material used in the following description of the species was fixed in 1939, either by Mare (formalin) or by myself (alcohol after narcotization).

Magelona alleni n.sp.

Adult specimens in a moderate state of expansion about 40 mm long by 1 to 1·5 mm wide anteriorly (but see p. 625) tapering gradually to the anus; a constriction at the ninth setiger. First nine setigers bristle-bearing, the following setigers with hooded hooks only. Total number of setigers about 70. Prostomium (Fig. 1, C) eyeless, spatulate, wider than long especially in contraction (Fig. 1, A) and with two elongate low dorsal ridges broadened posteriorly, on either side low raised areas. The anterior border of the prostomium is almost straight transversely, short and without horns. The proboscis when everted is globular, ridged. On each side of the mouth, ventral to the postero-lateral corners of the prostomium, there arises a long tentacle,* transversely wrinkled and bearing for much of its length distally a large number of densely crowded papillae, elongate and slightly capitate. When a tentacle has been broken off the postero-lateral border of the prostomium bends inwards ventrally, so reducing the apparent width of the prostomium viewed dorsally (Fig. 1, C, right side). First eight setigers with relatively strong dorsal and ventral winged bristles (Fig. 1, H) springing in bundles of often eight to sixteen bristles from the bases of pointed lamellae (Fig. 1, C, D and F). Some bristles are double-winged; in many, as in the one drawn, the narrow secondary wing is absent. The dorsal lamellae are flattened antero-posteriorly, the ventral lamellae dorso-ventrally, the planes of flattening thus being at right angles to one another. These first eight pairs of parapodia are situated at the anterior borders of the segments. The ninth parapodia and bristles are similar in structure but are smaller and shorter and the segment bearing them is very short with ill-defined limits: it is also much narrower than those in front and behind, producing a marked constriction of the body at this region (Fig. 1, D, E). The reduction in girth of this ninth setiger is largely responsible for the displacement of the noto- and neuropodia, especially the latter, towards the mid-dorsal and mid-ventral lines respectively.

The parapodia of the tenth and all succeeding setigers are situated near the middle of the segment to which they belong. They bear only hooded hooks, each with one main tooth surmounted by two smaller teeth (Fig. 1, I–K). There are about twelve hooks in two facing groups in each noto- and neuropodium (Fig. 1, G), except posteriorly where the numbers are less. The rows of hooks reach to within short distances of the mid-dorsal and mid-ventral lines. Between the dorsal and ventral rows of hooks of each parapodium there is a large leaf-like dorsal lamella and a much smaller ventral lamella, both lamellae flattened antero-posteriorly. Ventrally and medianly to the ventral rows of hooks there is a very small protuberance, or rudimentary ventral cirrus. Except for the incompletely developed parapodia at the extreme posterior end all the parapodia from the tenth onwards are closely similar. The body ends in a clearly defined pygidium with the anus between two short stumpy cirri (Fig. 1, B).

On both dorsal and ventral surfaces of the body there are in the anterior region a pair of longitudinal grooves. At the region of the ninth setiger these grooves merge

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* Other writers variously term these organs tentacles, palps, etc. My own choice of term should not be considered an expression of opinion about their homology.

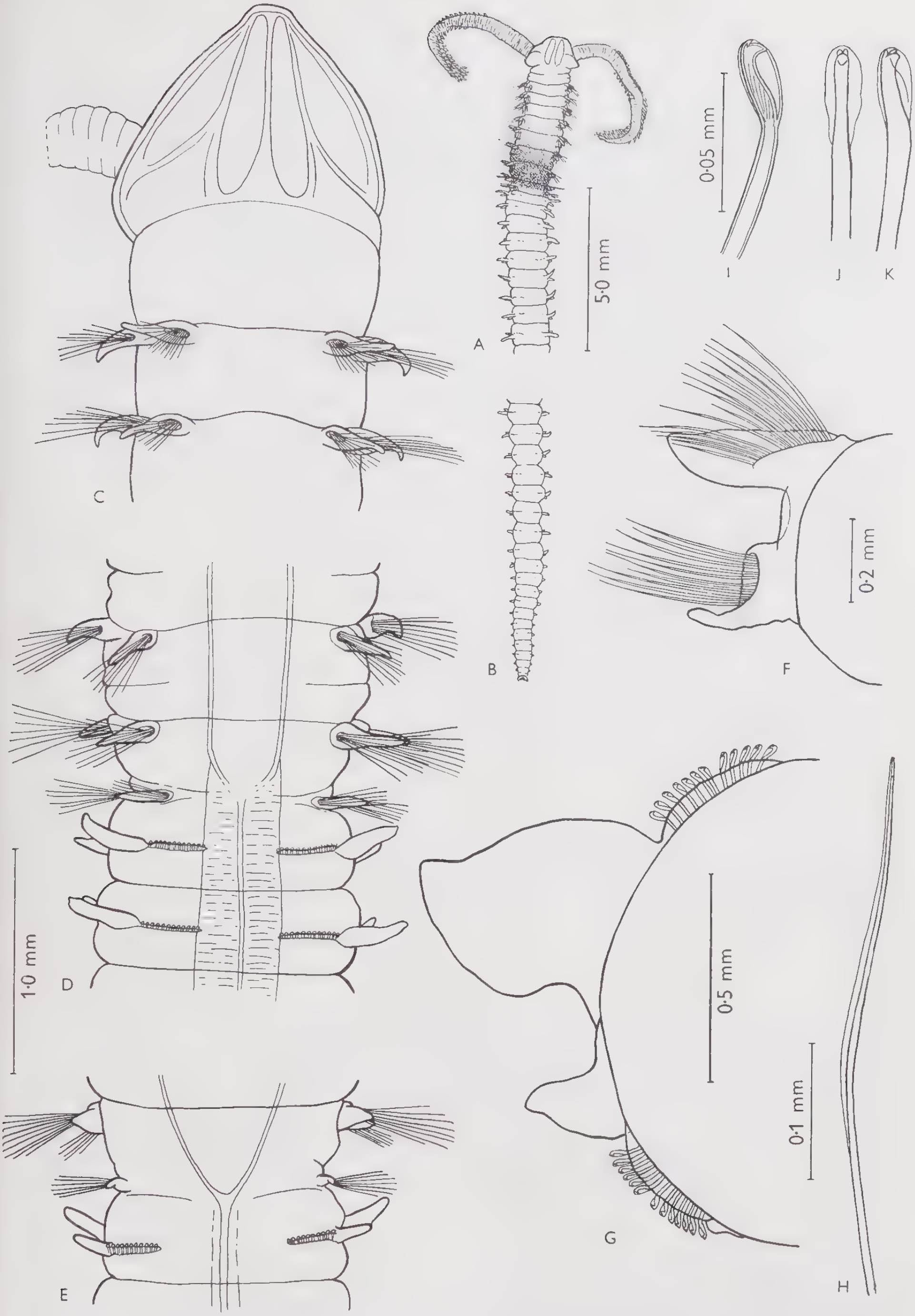

Fig. 1. *Magelona alleni* n.sp. A, dorsal view of the anterior portion of a partially contracted specimen showing band of reddish pigment. B, dorsal view of the posterior portion of another specimen. C, dorsal view of the head and first two setigers of a third specimen fixed with little or no contraction. D, dorsal view of the 7th–11th setigers of the same specimen. E, ventral view of the 8th–10th setigers of the same specimen. F, view from in front of 5th parapodium from a fourth specimen. G, 31st parapodium of the fourth specimen. H, winged bristle. I, hooded hook in side view. J, another hook in front view. K, a third hook in oblique front view.

into single dorsal and ventral median grooves (Fig. 1, D, E), which continue to the anus. The depth of the grooves varies greatly with the state of contraction of the body wall muscles and they are often obliterated.

In life the general colour is yellowish with a prominent band of deep reddish pigment encircling the body in the anterior region (Fig. 1, A). It occurs from the fourth or fifth to the eighth or ninth setiger and is densest on the sixth to eighth setigers, shading away in front and, more steeply, behind. This pigment persists in formalin and alcohol-preserved specimens, but is then often lighter and somewhat brownish. In the specimen fixed in hot Bouin's Fluid and preserved in alcohol the pigment disappeared altogether. There is some indication that it slowly fades in specimens preserved in alcohol and kept in the light.

The following characters in combination are sufficient to distinguish *alleni* from all other known species: (1) the absence of prostomial horns, (2) the unmodified ninth parapodia, (3) the large notopodial and small neuropodial lamellae of the tenth and succeeding setigers, (4) the girdle of red pigment between the fourth and ninth setigers, but this pigment may be destroyed by some fixatives.

Habitat: muddy bottoms offshore extending into cleaner fine sand. Appears to form mucus-lined burrows that may be described as fragile tubes.

Distribution: Plymouth, Clyde (see p. 625), Dogger Bank (see p. 625), Quiberon Bay, N.W. France (specimens collected by N. A. Holme, 19.6.55), possibly Atlantic coast of Morocco (see p. 624). Probably a temperate-water species of the north-eastern Atlantic.

A specimen, portions of which are drawn in Fig. 1 C–E, has been deposited in the British Museum (Natural History) and given the holotype number 1958.5.2.1. It comprises an anterior portion of fourteen setigers, 7·5 mm long, and is without tentacles except for a stump on the left side; the red band has bleached in fixation. Other specimens have also been deposited and given the paratype numbers 1958.5.2.2/10.

The species is named in honour of the late Dr E. J. Allen, F.R.S., former Director of the Plymouth Laboratory, who did so much to advance the study of polychaetes at Plymouth and who contributed notably to scientific knowledge of them.

A RE-ASSESSMENT OF *MAGELONA CINCTA* EHLERS

Magelona cincta was described and figured by Ehlers (1908, pp. 111–12, Taf. xv, figs. 9–12) from a single anterior portion obtained by the 'Valdivia' (1898–99) from a depth of 40 m in Algoa Bay, South Africa. At that time only four other species of *Magelona* had been described (*papillicornis* F. Müller, 1858; *longicornis* Johnson, 1901; *obockensis* Gravier, 1906; *rosea* Moore, 1907) and one of them, *obockensis*, was subsequently regarded by McIntosh (1925) and Monro (1933) as a variety of *papillicornis*. *M. cincta* was the first species to be discovered with a strongly marked band of pigment encircling the body. Subsequently, *japonica* Okuda (1937) was described as having a deep purple band of pigment from the 5th to 8th setigers, that is in the same region as the red pigment in *cincta* and now in *alleni*. The markedly horned prostomium, parapodial structure and other features clearly separate *japonica* from *alleni* and from *cincta*. Ehlers's figures, especially the coloured one, bear a strong resemblance to *alleni*, but his drawings and description are not entirely satisfactory (Wesenberg-Lund, 1949, p. 330, is in agreement) and lack proper figures of the parapodia. I could never satisfy myself that our species was in fact *cincta*; neither could I be quite sure on the basis of Ehlers's work that it was anything more than a variety.

Day (1957) has recorded *M. cincta* from South Africa. He has kindly sent me three of his specimens; one of them a short anterior end of about seventeen setigers from a depth of 15 m in Mossel Bay some 200 miles west of Algoa Bay, the type locality. The other two specimens, each of about forty setigers, came from intertidal sandy mud on Inhaca Island, Delagoa Bay, some 800 miles along the coast north-eastwards of Algoa Bay. These specimens are undoubtedly the same species and I am in agreement with Day's identification with *cincta*. They agree closely with Ehlers's description and figures, except that in all three the anterior extremity of the prostomium has at each side of the almost straight front edge much more pronounced corners—almost horns (Fig. 2 A–C)—than Ehlers indicates in his drawings. We are entitled to conclude that Day's specimens are true *cincta* and that they can be used to add to and amend the original description of the species by Ehlers. This will now be done so far as is necessary to define *cincta* more precisely and to distinguish it clearly from *alleni*.

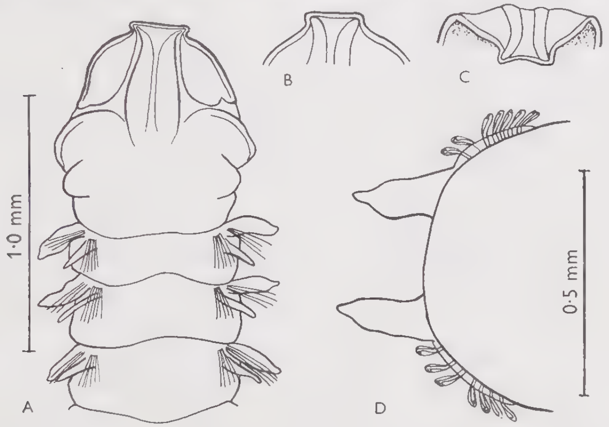

Fig. 2. *Magelona cincta* Ehlers. A, dorsal view of the head and first three setigers of a specimen from Mossel Bay, South Africa. The anterior third of the prostomium is bent upwards a little, giving a slightly foreshortened view of this end. The bristles are more numerous than drawn. B and C, anterior extremities of two specimens from Delagoa Bay, South Africa, both in dorsal view but C has the tip bent over backwards, revealing the ventral surface. D, 39th parapodium.

The prostomium of *cincta* is in preserved specimens (Fig. 2A) about as long as broad, terminating anteriorly in a straight edge with pronounced corners, or very short horns. Two medianly placed low ridges extend anteriorly into the corners and are separated by shallow troughs from slightly convex areas on either side. There are no eyes. One specimen retains one tentacle; it is at least twice as long as that indicated by Ehlers. The tentacle bears long and densely crowded papillae on one face for most of its length; the distal portion is slender, tapering gradually almost to a point and has evidently been fixed in a state of extension.

The anterior setigers all bear winged bristles only, unmodified on the ninth setiger. The bristles are relatively less robust than are those of *alleni*, a feature immediately obvious when similar-sized specimens of the two species are placed side by side. The parapodial lamellae of the first three or four setigers (Fig. 2A) are larger than those of succeeding setigers and they are quite small on the ninth. The neuropodial lamellae are larger than the notopodial. A band of brownish red (in alcohol) pigment encircles the body from the 5th to 8th setigers. The body is slightly constricted at the short and ill-defined 9th setiger.

The 10th and all succeeding setigers carry hooded hooks only. Each hook has one main tooth surmounted by two small teeth as in *alleni*. Ehlers did not notice that what appears as a single small upper tooth in side view is seen to be two teeth side by side when the hook is examined from behind or from in front. Between the dorsal and ventral rows of hooks there are in each parapodium two moderate-sized lamellae of about equal size (Fig. 2D). On the 10th to about the fifteenth setiger these lamellae are a little broader than on the setigers behind, but on the whole there is little difference anywhere and all the parapodia of the posterior region as far back as the 40th setiger (where the longest specimen ends) are closely similar. This equality in size between the neuro- and notopodial lamellae is a striking difference from *alleni* and is in itself sufficient distinction between the two species.

The two specimens from Delagoa Bay, each of about forty setigers and incomplete posteriorly, are both about 15 mm long and about 0·7 mm wide. Day informs me that his most westerly specimens, three in number, are from False Bay and were living in green mud at a depth of 43 fathoms.

The type specimen

Since the foregoing was written I have received on loan from the Zoologisches Museum, Berlin, to whose authorities and Dr G. Hartwich in particular I should like to express my gratitude, the type specimen described and figured by Ehlers (1908). It enables me to confirm that Day's specimens are definitely *cincta*. The type specimen is a fragment from a somewhat larger worm than those sent by Day but is very much smaller than full-grown *alleni*. Ehlers's specimen, as he recorded, is about 16 mm long by 1 mm wide and consists of a head and nineteen setigers. The right tentacle is missing; part of the left one is present and is clearly broken short. The specimen shows every indication of having been partially flattened. This has resulted in the extruded proboscis, which in *Magelona* worms normally forms a ventral bulbous protrusion, being compressed and forced to extend forwards almost to reach the anterior end of the prostomium, as seen in Ehlers's figures (taf. XV, figs. 9, 10*a*) and in my Fig. 3A drawn with the aid of a camera lucida. The prostomium has been so flattened on top that the dorsal ridges, clearly visible in Day's specimens, can only just be distinguished. Ehlers's artist, when drawing his fig. 10*a* (but not his fig. 9) misinterpreted the structural relations

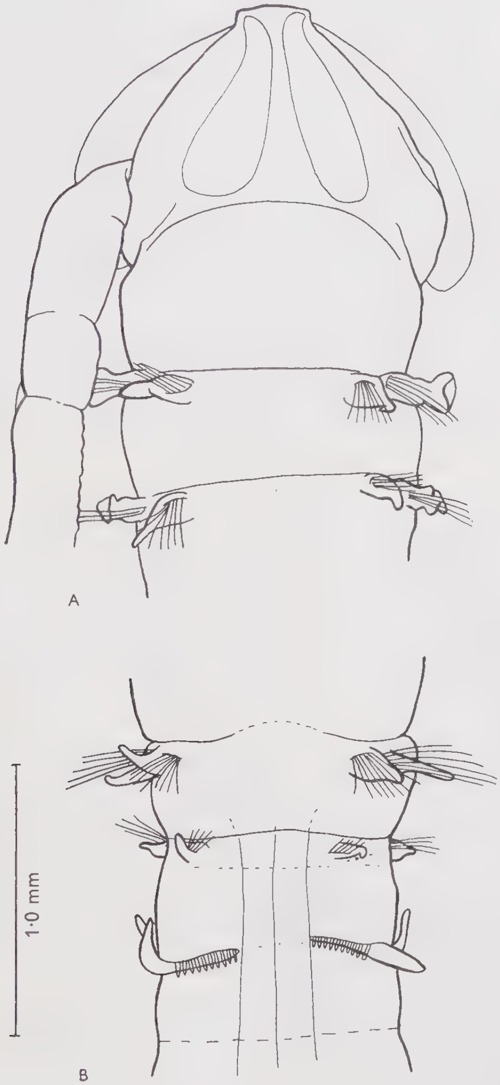

Fig. 3. The type specimen of *Magelona cincta* Ehlers in dorsal view. The bristles are more numerous than drawn. A, head and first two setigers. B, 8th–10th setigers.

between the postero-lateral corners of the extruded proboscis and the overlying prostomium, for he makes the proboscis tissue merge with the lateral walls of the body. The text shows that Ehlers himself was not clear in his own mind concerning the morphology of these parts. The artist has also 'smoothed out' the forwardly projecting anterior tip of the prostomium with its marked corners, which whilst not quite as prominent as in Day's specimens (no doubt due to the flattening) are none the less present, and have been carefully traced in my Fig. 3A. The constriction of the trunk at the 9th setiger is not as great as is represented in Ehlers's fig. 10B, which should be compared with my Fig. 3B, and the intersegmental grooves are by no means as distinct as Ehlers's artist shows them. He has represented the posterior boundary of the 10th setiger as being immediately behind the parapodia, but this boundary is very difficult to see (no doubt due to the flattening). It undoubtedly lies further back, as in Day's specimens,* and it can just be made out in the position indicated by my broken line in Fig. 3B. Some other reinterpretations of details will be noticed when Ehlers's figures and mine are compared.

I have confirmed that the hooks on Ehlers's specimen bear two small teeth side by side above the main tooth, and that the parapodial lamellae of the posterior region are as in Day's specimens and agree with my Fig. 2D. The pigmentation of the 5th–8th setigers has become very faint but is still to be seen.

Distribution

If we except Fauvel's specimen from Morocco (see below), the known distribution of *M. cincta* Ehlers is confined to the south and south-east coast of Africa, from False Bay in the west to Mozambique in the north-east, a length of coast influenced by the warm Mozambique and Agulhas Current. It occurs in intertidal sandy mud and offshore mud to a depth of at least 43 fm.

VARIOUS SPECIMENS ATTRIBUTED TO *MAGELONA CINCTA* EHLERS

There remain for consideration several published records of *M. cincta* Ehlers and of specimens attributed to this species.

Fauvel (1936, p. 64) described a fragment of a *Magelona* from the Atlantic coast of Morocco which he identified as *cincta*, largely it seems on the basis of the coloration and some other characters which corresponded well with the description and figures of Ehlers. His fragment was an anterior portion of head and thirteen setigers measuring 8 mm long by 2 mm wide, a dimension which fits mature *alleni* and indicates a worm bigger than any known *cincta*.

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* In some specimens of *cincta* a transverse groove exists at parapodial level, depending on the state of contraction.

The prostomium was without horns, and 'les pieds portent une grande lamelle dorsale et une plus petite ventrale, et sont dépourvus de cirre'. This description almost perfectly describes the posterior parapodia of *alleni*. Fauvel's specimen may well be *alleni*, it cannot be *cincta*.

Clark & Milne (1955) found below tidal levels off the Island of Cumbrae in the Firth of Clyde some red-banded *Magelona* worms and, following a conversation with me, recorded them as *cincta*. Recently I have had an opportunity of examining several of their specimens and find that they are *alleni*.

From the British Museum (Natural History), through the kind offices of Mr N. Tebble, I have received on loan all their specimens attributed to *Magelona cincta*. They include one specimen (Reg. No. 1955.4.1.96) from the Morrumbene Estuary, Portuguese East Africa, which is indeed *M. cincta* Ehlers. It is of similar dimensions to those from Delagoa Bay (p. 622). A number of specimens (Reg. No. 1954.1.1.85/88) from the Dogger Bank, North Sea, are definitely *alleni*. Some further specimens (Reg. No. 1954.1.1.90/95) from Plymouth (collected originally by Mr N. A. Holme) are also *alleni*. It is interesting that several of these latter, all anterior ends, are fragments of larger worms than any in my own collection. One of them, comprising the head and seventeen setigers measures approx. 13 mm long by 2·5 mm where it is widest at the 6th–7th setigers; it was fixed in a state of contraction.

SUMMARY

A *Magelona*, common at Plymouth and previously provisionally identified as *M. cincta* Ehlers, is described as a new species and given the specific name of *alleni*.

M. cincta Ehlers is partially re-described from South African specimens and from the type specimen.

M. alleni n.sp differs from *M. cincta* Ehlers in several details including the shape of the prostomium and unmistakably in the structure of the parapodia of the posterior region. In *alleni* the notopodial lamellae are very much larger than the neuropodial while in *cincta* they are almost equal in size.

M. alleni occurs in the Firth of Clyde, on the Dogger Bank and in Quiberon Bay, N.W. France. A *Magelona* from the Atlantic coast of Morocco, described in the literature as *cincta*, is not *cincta* and is likely to be *alleni*. A wide distribution in the north-eastern Atlantic is indicated for this new species.

M. cincta Ehlers is known only from the southern and south-eastern coasts of Africa influenced by the warm Mozambique and Agulhas Current.

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ON SOME POGONOPHORA FROM THE NORTH-EAST ATLANTIC, INCLUDING TWO NEW SPECIES

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(Text-figs. 1–3)

In the course of dredging for the rich epifauna of the continental slope near 48° 30′ N., 10° W. (Southward & Southward, 1958*b*) in May 1957, several hauls were made by chance on a muddy bottom at 500–700 fm. depth. At the time, these hauls were examined only cursorily after sieving, and the contents immediately preserved. One of the hauls contained several damaged siliceous sponges, and a recent careful examination disclosed a number of pogonophore tubes entangled among the threads and spicules of the sponges.

TABLE 1. RECORDS OF POGONOPHORA FROM THE CONTINENTAL SLOPE

Date	Position	Depth (fm.)	Bottom deposit	Species of *Siboglinum* found
4. v. 57	48° 28′ N., 10° 04′ W.	700	Mud containing a little sand and foraminiferan shells	*S. atlanticum*, *S. inermis*, *S. ekmani*
	48° 32′ N., 10° 10′ W.	670–720	Mud and a few stones	*S. atlanticum* (tubes only)
	48° 31′ N., 10° 11′ W.	520–680	Mud and some gravel	*S. atlanticum* (tube only)
17. v. 58	47° 56′ N., 7° 57′ W.	340–350	Mud containing sand and foraminiferan shells	*S. ekmani*
	47° 50′ N., 7° 57′ W.	300–450	Mud containing sand and foraminiferan shells	*S. inermis*
	47° 50′ N., 8° 08′ W.	670–710	Mud and cretaceous rocks	*S. atlanticum*

Nearly forty tubes, of three types, were found in this haul, most of them containing the animal in a good state of preservation (Southward & Southward, 1958*a*). Further searches among our collections have brought to light some empty tubes taken in two other hauls on the same cruise of R.V. ‘Sarsia’. Once it was known that Pogonophora were present on the continental slope further dredging was arranged, and to date (June 1958) these interesting animals have been taken alive at three more stations (Table 1).

Although three species have been obtained, all belong to the genus *Siboglinum* Caullery. This was the first genus of the group to be described, and *S. weberi* remained for a long time the only known representative (Caullery, 1914, 1944). Later the Class name Pogonophora was suggested

(Johansson, 1937) for *Lamellisabella zachsi* Uschakov. *Siboglinum* was assigned to this group by Ivanov, who erected a new phylum of the Deuterostomia, Phylum Brachiata A. Ivanov, for the group (Ivanov, 1951, 1955). Investigations in the Pacific and Arctic oceans have revealed many new genera and species of pogonophores, while recently a species of *Siboglinum* has been discovered in deep water in the Skagerak (Jägersten, 1956; Ivanov, 1957; Kirkegaard, 1958). The Pogonophora are thus becoming well known, and their occurrence in the Atlantic Ocean proper is not unexpected.

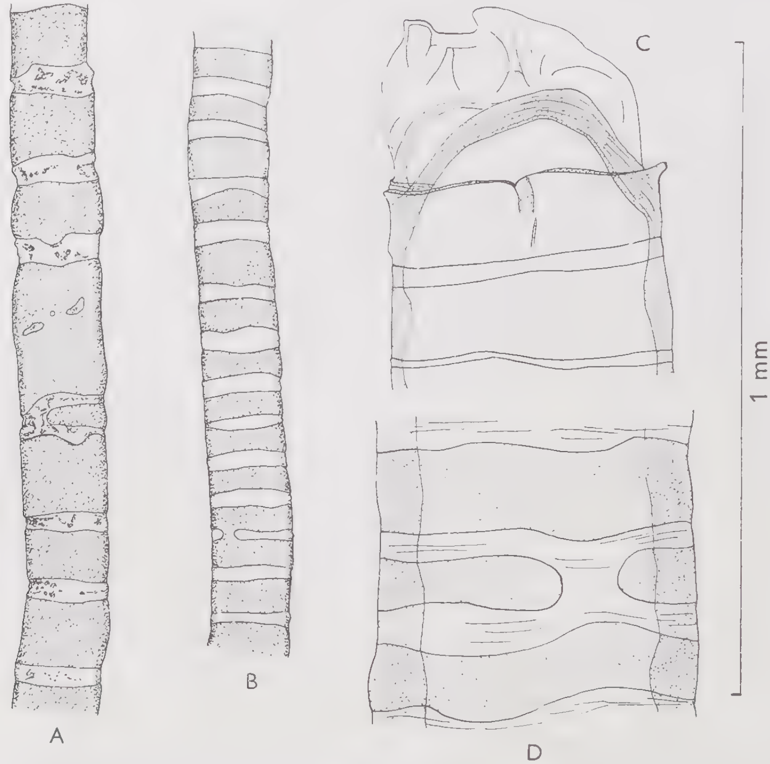

Fig. 1. Appearance of tubes: A, *Siboglinum inermis*; B, *S. ekmani*; C, anterior end of tube of *S. atlanticum*; D, middle of tube of *S. atlanticum*.

Of the three species included in this work, one is the Scandinavian species *S. ekmani*. The other two appear to be new, even though seven species of *Siboglinum* have already been described (Ivanov, 1957), omitting *S. weberi* which appears to include more than one species. The two new species are described first below, followed by some notes on our specimens of *S. ekmani*. Later we hope to deal with the biology of these animals, and to describe further species taken in only 80–90 fm. off the west coast of Ireland.

Siboglinum atlanticum sp.nov.

From twenty tubes of this species we obtained parts of fourteen animals. The two longest tubes were 40 and 32 cm long; most of the shorter ones were obviously incomplete. The diameter of each tube was about 0·42 mm near the anterior end, increasing to about 0·5 mm in the middle. The general colour was pale brownish grey, and the walls were ringed as shown in Fig. 1 D.

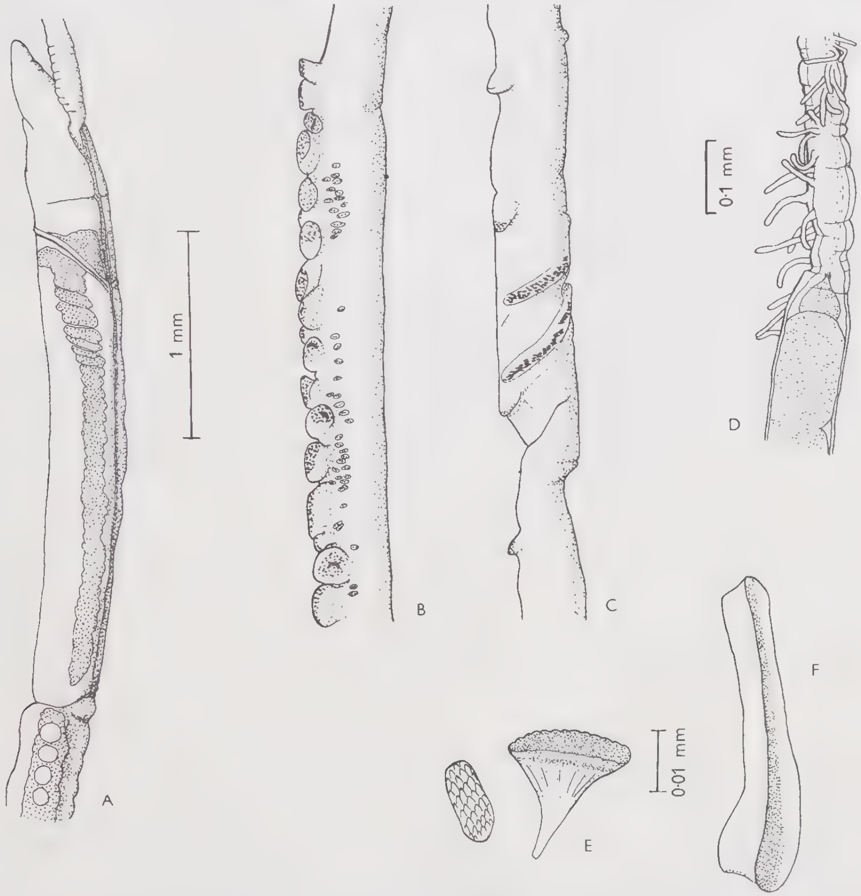

Fig. 2. *Siboglinum atlanticum*: A, anterior end, lateral view; B, part of region with papillae, lateral view; C, girdle region, lateral view; D, part of tentacle; E, uncini, surface and side view; F, platelet from one of the papillae, side view.

The anterior end is paler and in most cases is sealed by an extension of the tube lining (Fig. 1 C). Seven to 10 cm below this end is a brown mark, not more than 1 cm long, which may indicate the surface of the mud.

The two longest animals are both about 13 cm long, excluding the tentacle, which may reach 2 cm in length. The other specimens are anterior ends up to 4 cm long. The tentacle is usually coiled up in preserved material; it bears two rows of pinnules set close together and in many specimens it is distended with blood for all or parts of its length (Fig. 2D). The blood is bright red in living specimens and brown when preserved. The tentacle rises from a furrow on the ventral side (Fig. 2A), and its junction with the body is narrow. The dorsal lobe is small and pointed, with no perceptible groove between it and the rest of the protosoma, but there is a slight and incomplete groove between the proto- and mesosoma. The length of the protosoma varies from 0·68 to 0·87 mm in length, while the mesosoma varies from 2·4 to 2·9 mm. The ventral furrow continues all along the mesosoma and a shallow dorsal furrow is usually present on both protosoma and mesosoma.

The 'bridle' or cuticular ring on the mesosoma is narrow and is set in a slight groove; it is not complete on either the dorsal or ventral side. Two small patches of epidermal glands are present in front of the bridle (Fig. 2A), and behind it lateral bands of the same type of gland cells extend almost to the end of the mesosoma.

The metasoma is divided up into several regions. The first or 'metameric' region (Jägersten, 1956) bears two rows of large glands on the ventral side with a deep furrow betwen them, and varies from 15–20 mm in length. It is followed by a region 40–50 mm long, bearing a few papillae and containing the gonads and genital products. The genital region precedes a short region with a large number of papillae (up to 34,) each bearing a dark platelet (Fig. 2B, F). Of our two longest specimens, one has two girdles of uncini (Fig. 2C, D) and the other three. The girdles lie about 8 mm from the end of the genital region, and are followed by a narrow region with a few papillae, extending about 28 mm.

S. atlanticum seems clearly distinct from *S. caulleryi* and *S. plumosum*, the largest of the species described by Ivanov (1957).

Siboglinum inermis sp.nov.

Five tubes, each containing an animal, were collected in 1957, and fifteen tubes, some empty, were collected in 1958. The longest tube was 65 mm long and may not have been complete, although others about 50 mm long appear to be complete. The middle region is dark brown with wide rings (Fig. 1A). These rings are almost opaque, and it is difficult to see the animal inside. Both ends of the complete tubes are colourless. The diameter of the tube ranges from 0·13 to 0·18 mm. The complete animal varies from 13·5 to 25 mm long, with a tentacle up to 3 mm long. The tentacle, in preserved and living specimens, is entirely without pinnules, and the specific name refers to this. The anterior part of the body varies from 0·70 to 0·96 mm in length, of which the protosoma forms 1/5 to 1/4 (Fig. 3A). The narrow bridle is complete ventrally, but broken dorsally; a faint longitudinal groove runs along the ventral side of the mesosoma (Fig. 3 A, B). It is difficult to distinguish epidermal glands on the mesosoma, but there seem to be at least two small patches just behind the bridle. The metasoma is short compared with that of *S. ekmani*; in one complete female it is 14 mm long, of which 5 mm carries metameric glands, 3 mm contains ovaries, 2 mm has a few papillae (without platelets), which are followed by the girdles and about 4 mm with a few papillae. Of six specimens with complete girdle regions, two have three girdles and four have two. Each girdle is made up of a single row of uncini (Fig. 3 C, D).

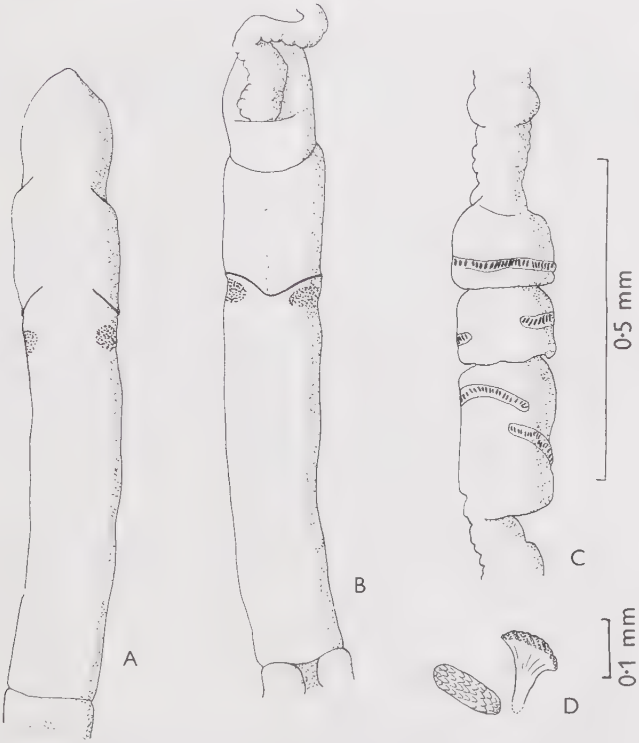

Fig. 3. *Siboglinum inermis*: A, anterior end, dorsal view; B, anterior end, ventral view; C, girdle region; D, uncini, surface and side view.

This species differs from *S. ekmani* in the type of tube, the diameter, the proportions of the body and in having a tentacle without pinnules.

Siboglinum ekmani Jägersten

The specimens described by Jägersten (1956) were incomplete, but our specimens agree with his description in the form (Fig. 1 B) and size of the tube, in the proportions of the body and in having two rows of pinnules on the tentacle. In our specimens the length of the longest tube was 10·5 cm, and the complete animal would appear to be about 30 mm long. The 'endobody' noted by Jägersten must have been produced by damage or preservation, since our specimens have a girdle region similar to *S. inermis*, except for the arrangement of the girdles. Each is composed of one row of uncini; the first and second are less than 0·2 mm apart, while the third is at least 1·5 mm behind the second. In one specimen there is a further 10 mm of body behind the girdles.

SUMMARY

Three species of Pogonophora are recorded from the Atlantic at depths of 710 up to 340 fm. They belong to the genus *Siboglinum*, two of them being new species, *S. atlanticum* and *S. inermis*, which are here described. The third is *S. ekmani* Jägersten.

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ON THE OCCURRENCE AND BEHAVIOUR OF TWO LITTLE-KNOWN BARNACLES, *HEXELASMA HIRSUTUM* AND *VERRUCA RECTA*, FROM THE CONTINENTAL SLOPE

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(Plate I and Text-figs. 1–5)

The fauna of the continental shelf and slope between Ireland and Spain has been described by Le Danois (1948), but very little faunistic work has been carried out in the area recently. Since R.V. 'Sarsia' was brought into service at Plymouth there have been several opportunities of investigating that part of the slope lying to the south-west of the British Isles. It has been found that, in addition to beds of coral, quite extensive exposures of rocks, stones and gravel occur between 200 and 1000 fathoms. There is a rich epifauna in this area and barnacles are one of the dominant groups.

Only two species of barnacles are abundant. Both of them have been found, either as live specimens or dead shells, at many of the stations investigated. They are *Hexelasma hirsutum* (Hoek) and *Verruca recta* Aurivillius. We will first describe these species and their associated fauna, and then discuss their behaviour, as observed in the laboratory.

We are indebted to Dr L. H. N. Cooper for advice in this work, and to Dr J. P. Harding for facilities at the British Museum (Natural History). Thanks are due to Captain C. A. Hoodless and the crew of R.V. 'Sarsia', for their patience during many days of fruitless dredging, and to Dr D. Atkins, whose request for dredging on the slope led to the discovery of the barnacles.

Hexelasma hirsutum (Hoek)

Balanus sp. undescribed, Jeffreys, 1878.
B. hirsutus Hoek, 1883.
B. hirsutus Hoek, Gruvel, 1920.

Hexelasma hirsutum was first described by Hoek from material collected by H.M.S. 'Triton' in the Faroë–Shetland Channel, Station 10, 1882. The two specimens are now in the British Museum. Hoek assigned the species to the genus *Balanus*, and it was not until other related species were found that their

TABLE 1

Collection	Station	Depth	No. of specimens	Substratum	Author, or present location of material
			Hexelasma hirsutum		
'Porcupine'	48° 31′ N., 10° 03′ W.	690 fm	1	Shell of living brachiopod	Jeffreys, 1878 (as *Balanus* sp.)
'Triton'	59° 40′ N., 7° 21′ W.	516 fm.	2	*Cidaris* spine	Hoek, 1883 (as *B. hirsutus*)
Monaco	38° 31′ N., 26° 49′ W.	845 m	2	—	Gruvel, 1920 (as *B. hirsutus*)
Monaco	Azores area	—	3	Telegraph cable	Gruvel, 1920 (as *B. hirsutus*)
'Monarch'	48° 04′ N., 9° 23′ W.	1000 fm.	24	Telegraph cable	Specimens in British Museum
'Monarch'	48° 02′ N., 9° 25′ W.	930 fm.	6	Telegraph cable	
'Monarch'	48° 02′ N., 9° 27′ W.	900 fm.	10	Telegraph cable	
'Marie-Louise Mackay'	51° 23′ N., 11° 32′ W.	210 fm.	3	Telegraph cable	Specimens in authors' possession
'Sarsia' 1956/4	48° 33′ N., 10° 05′ W.	570–770 fm.	>60 and old shells	Rocks, gravel, dead coral, living brachiopod	
'Sarsia' 1956/8	47° 30′ N., 7° 20′ W.	870–970 fm.	Old shells only	—	
'Sarsia' 1957/1	48° 34′ N., 10° 0′ W.	570–700 fm.	>90 and old shells	Rocks, gravel, antler	
'Sarsia' 1957/1	48° 33′ N., 10° 01′ W.	580–680 fm.	Old shells only	—	
'Sarsia' 1957/1	48° 31′ N., 10° 11′ W.	520–680 fm.	Old shells only	—	
			Verruca recta		
Monaco	Azores area (8)	861–1385 m	10	Stones, rocks, coal and dead coral	Aurivillius, 1898; Gruvel, 1920
'Talisman'	Azores area (1)	960–990 m	1	Coral	Gruvel, 1902
'Travailleur'	—	2018 m	1	Coral	Gruvel, 1902
Monaco	28° 04′ N., 16° 49′ W.	1340–1530 m	1	—	Gruvel, 1920
'Sarsia' 1956/4	48° 33′ N., 10° 05′ W.	570–770 fm.	13	Stones, dead coral, shell fragments	Specimens in authors' possession
'Sarsia' 1956/8	47° 30′ N., 7° 28′ W.	550–600 fm.	1	Coral	
'Sarsia' 1956/8	47° 38′ N., 7° 28′ W.	710–750 fm.	8	Small stones, coral, clinker	
'Sarsia' 1956/8	47° 30′ N., 7° 20′ W.	870–970 fm.	2	Coal, coral	
'Sarsia' 1957/1	48° 42′ N., 9° 48′ W.	180–200 fm.	1	Shell	
'Sarsia' 1957/1	48° 33′ N., 10° 01′ W.	580–680 fm.	Old shells only	Stones, coral	
'Sarsia' 1957/1	48° 34′ N., 10° 00′ W.	570–700 fm.	12	Stones, coral	

affinity with the Chthamalidae was recognized, and the new genus *Hexelasma* erected (Hoek, 1913). Table 1 shows the older and the more recent records of *H. hirsutum.* Jeffreys's record is almost certainly this species, even though the specimen was not described (Jeffreys, 1878, p. 414). Its occurrence at the 'Porcupine' station on the shell of a living brachiopod (*Hispanirhynchia cornea* (Davidson), recorded by Jeffreys as *Rhynchonella sicula* Seguenza) has been repeated in one of the 'Sarsia' hauls; a living specimen of the same brachiopod was taken with a live *H. hirsutum* on one valve and a live *Verruca recta* on the other valve.

The known range of *Hexelasma hirsutum* is from the Faroë–Shetland ridge to the Azores. A related species, *H. americanum* Pilsbry, found on the western side of the Atlantic, appears to differ in external features and in the shape of the tergum (Pilsbry, 1916).

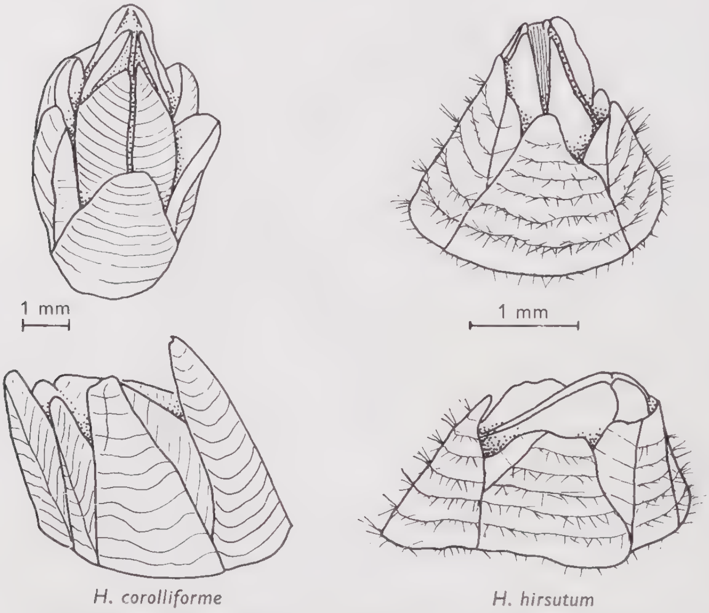
Description

The species was defined by Hoek as follows: 'Basis membranous; radii absent; parietes not permeated by pores; articular ridge of tergum broad, but not extending so far downwards as in *Balanus corolliformis*. Carinal margin of tergum much shorter than the basal margin. Membrane lining the growth ridges with distinct spines.' However, of these characteristics, the absence of radii, the non-porous parietes, and, apparently, the non-calcareous basis apply to all the known species of *Hexelasma*, and must be considered generic (cf. Hoek, 1913; Pilsbry, 1916). Clearly there is considerable difficulty in separating the species of *Hexelasma*. As far as we are concerned at present, it is only required to show the distinction between *H. hirsutum* and the others. It has, however, been necessary to go into this matter fully, since Nilsson-Cantell (1930) believed *H. hirsutum* to be the young form of *H. corolliforme*. We have therefore re-examined the type specimens of *B. corolliformis* and *B. hirsutus* in the British Museum, as well as Nilsson-Cantell's specimens of *H. corolliforme* in the 'Discovery' collection.

H. hirsutum can be distinguished from other species by its markedly conical or tent-like shape (Pl. 1, fig. 1). Even on restricted substrata, such as sea-urchin spines and branches of coral, the diameter of the orifice is much smaller than the maximum diameter of the specimen (usually less than half). In normal uncrowded specimens from stones the diameter of the orifice is about two-fifths to one-third of that of the base, and the compartments do not diverge from one another. In these characters this species differs strongly from *H. corolliforme*. We have examined small specimens of both species (down to 1 to 2 mm in diameter in *H. hirsutum*) and find the shape still distinctive (Text-fig. 1).

The difference in shell form is clearly demonstrated by the rostral compartment, which is triangular in *H. hirsutum* and elongate in *H. corolliforme* (Text-fig. 2). The rostra of both old and young *H. hirsutum* have an index (height/width) of 0·8–0·9, while the type and other specimens of *H. corolliforme* have an index of 1·2–3·1. The other main character distinguishing *H. hirsutum* from *H. corolliforme* and most other species is the shape of the tergum.

	Length carinal margin/ length of basal margin	Length articular ridge/ length of basal spur
H. hirsutum	1/1·4	1/1·5
H. corolliforme	1/1·1–1·2	1/1·1–1·2

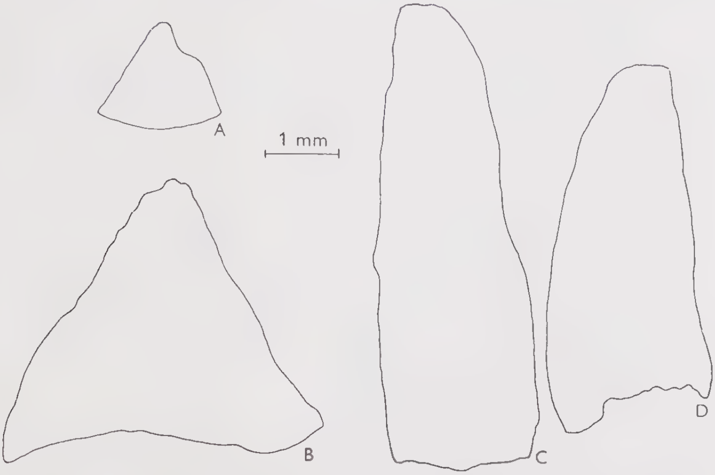


Text-fig. 1. Small specimens of *Hexelasma corolliforme* and *H. hirsutum*. Above, oblique view from rostral aspect; below, side views with rostra adjacent.

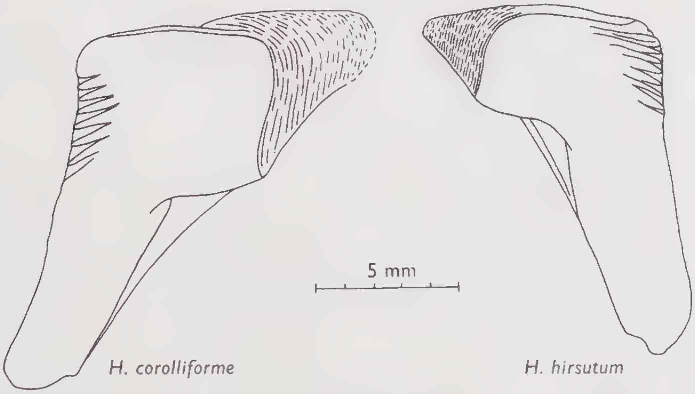
These measurements show that, in *H. hirsutum*, as reported by Hoek, the tergum is more elongate from apex to spur and the articular ridge is relatively shorter (Text-fig. 3).

There appear to be few other differences between the species of *Hexelasma*, and we have not been able to find any differences between the mouthparts of *H. hirsutum* and *H. corolliforme*.

All our specimens are conical in shape and fully hirsute all over (Pl. I, figs. 1–5). The edges of the compartments are reflexed under the basis for 1–2 mm or less depending on size. On removal from the substratum a distinct 'scar' can be seen. This is possibly the same as the thin calcareous basis reported by Pilsbry (1916) for another species of the genus. Only the remains of the reflexed edges of the compartments show strong effervescence with acetic acid. The vaguer white patches in the middle of the scar partly disappear in acid and may be weakly calcified. The scar shows distinct growth rings, and must therefore be formed by the reflexed margins of the compartments rather than by the basal membrane. It cannot be regarded as a true calcified basis such as is found in species of *Balanus*.



Text-fig. 2. Outline drawings of rostra of *Hexelasma hirsutum* (A and B), and *H. corolliforme* (C and D).



Text-fig. 3. Terga of large specimens of *Hexelasma*, inner side, showing articular ridge.

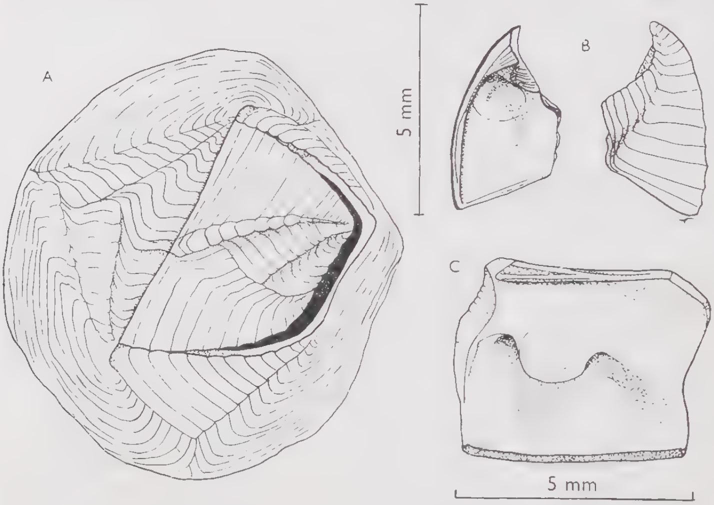
Verruca recta Aurivillius

Verruca recta Aurivillius, 1898.
V. linearis Gruvel, 1902.
V. recta Aurivillius, Gruvel, 1920.

This species was first described by Aurivillius (1898), who found it among material from the Azores region (see Table 1). It was next encountered by

Gruvel (1902) in another collection from the Azores. He named it *V. linearis*, believing his specimens to be specifically distinct from those of Aurivillius. However, when he examined the Monaco collections he decided that his earlier specimens were, after all, *V. recta* (1920). By this time specimens had been collected at further stations in the Azores regions and also at one station off the Canary Islands.

Verruca magna Gruvel, 1920, was described from a single specimen collected in the Gulf of Gascony (depth 1480 m) and it seems to be very similar to *V. recta*, differing only in its larger size and somewhat distorted shell.



Text-fig. 4. *Verruca recta.* A, whole shell, viewed from above; B, inner and outer aspects of movable scutum; C, inner aspect of fixed scutum.

V. coraliophila Pilsbry, 1916, is another species strongly resembling *V. recta*, occurring near the Bahamas in depths of 277–434 fm. It resembles *V. recta* in both morphology and habitat, and also occurs with a species of *Hexelasma*.

Recent discoveries of *V. recta* are listed in Table 1. Its known geographical range is from 48° 42′ N. to 28° 4′ N. on the east side of the Atlantic, and its depth range is 200–1000 fm.

Description

The external characters of *V. recta* were described by Aurivillius (1898) and Gruvel (1902, 1920); we will here describe certain variations in these features and also some of the internal features.

The appearance of the whole animal is shown in Text-fig. 4A; the operculum is formed of either the right of left tergum and scutum, and we have about equal numbers of specimens of each type. The movable scutum (Text-fig. 4B) has two large articular ridges and a third very narrow ridge, which is not visible from above in about half our specimens. The basal edges of the fixed plates are reflexed inwards as in *V. coraliophila* (Pilsbry, 1916), and there is a tongue-shaped adductor ridge protruding from the inner side of the fixed scutum (Text-fig. 4C). Neither of these characters was noted by Aurivillius or Gruvel; they indicate that *V. recta* belongs to the same subgroup of the genus as *V. coraliophila*, and that the two species may be synonymous.

The mouthparts are of the usual *Verruca* type, with no distinctive characters. The proportions of the rami of the first three pairs of cirri are of more help. The first cirrus has equal rami; the second has an anterior ramus about two-thirds the length of the posterior; in the third cirrus the anterior ramus is about four-fifths the length of the posterior. The caudal appendages are shorter than in other species of *Verruca*, being only one-fourteenth of the length of the sixth cirrus, and about three-quarters the length of its protopodite. In a large specimen each appendage had only seven segments. Pilsbry (1916) has recorded measurements of the cirri and caudal appendages for several species of *Verruca*, though not for *V. coraliophila*. Of these species the one with the most nearly corresponding measurements is *V. halotheca* Pilsbry, a Pacific species belonging to the same subgroup of the genus, and one which also resembles *V. recta* in both external features and habitat.

HABITAT AND ASSOCIATED FAUNA

The depths at which *Hexelasma hirsutum* and *Verruca recta* have been found, and the nature of the substrata on which they occur, are included in Table 1. Both species have been found on the same variety of substrata, including igneous and sedimentary rocks and living or dead shells of other animals, though only *Hexelasma* has been recorded from telegraph cables. Presumably they will settle on almost any exposed solid object if other conditions are favourable.

The two species differ somewhat in depth range. *Verruca* was found from just under 200 fm. to about 1000 fm., although most abundant at the 500–700 fm. level. It appears to be the more widespread, occurring alive at more of the 'Sarsia' stations than *Hexelasma*. The latter, with one exception, has been found only between 500 and 1000 fm. The exception is material from a cable, and it seems possible that the cable was hauled up from deeper water than that beneath the ship. *Hexelasma* is very abundant in the vicinity of the original 'Porcupine' station. The sea bed in this region of the continental slope is not particularly steep, but is very irregular. The echo-sounder records show the multiple criss-crossing bottom traces characteristic of submarine valleys, and it seems possible that the steep sides of these valleys may offer a very favourable habitat.

At the 500–700 fm. level, where both *Hexelasma* and *Verruca* are abundant, there is a characteristic associated fauna. Sponges, probably *Hymedesmia* sp., cover some of the rocks and sometimes the barnacles themselves. Attached to the rocks and stones, among the barnacles, are several species of solitary corals; two or three as yet unidentified species of serpulid worms; and the holothurian *Psolus squamatus* Koren. Mobile animals found in the same dredge hauls included the polychaetes *Eunice pennata* (O. F. Müller) and *E. oerstedii* Stimpson, the decapod *Munida tenuimana* Sars*; the echinoderms *Ophiacantha* sp., *Ophiactis* sp., and *Stereocidaris ingolfiana* Mortensen; and the brachiopods† *Hispanirhynchia cornea* (Davidson) and *Dallina septigera* (Lovèn).

At one of the deeper stations (47° 30′ N., 70° 20′ W., 870–970 fm.) at which *Verruca* was found in the living condition, the fauna was rather different. Living and dead branches of the coral *Anisopsammia rostrata* (Pourtales) were abundant. Also present were *Munida microphthalma* A. Milne-Edwards*; the echinoderms *Ophiacantha* sp., *Ophiactis* sp., *Korethraster hispidus* Wyv. Thomson and *Hypsechinus* sp.; and the brachiopod *Platidia* sp.

At the shallower station (48° 42′ N., 9° 48′ W., 180–200 fm.) at which only *Verruca* was obtained, the fauna was characteristic of the coral region of Le Danois (1948), with *Lophohelia*, *Dendrophyllia*, and *Caryophyllia*, as well as several decapods and eunicid worms.

BEHAVIOUR

Some notes on the behaviour of *Hexelasma hirsutum* were made on the material collected in 1956. Regular beating of the cirri, such as occurs in most other species of barnacles, was never seen; it was found that water movement was necessary to induce extension of the cirri, which were fully protruded from the shell only within a restricted range of temperatures (Southward, 1956). These findings have been partly confirmed and partly amended by experiments on the 1957 material, as described below.

Water currents

The influence of water movement was studied at several temperatures (Table 2). Full extension of the cirri was induced only by water currents in excess of 1 cm/sec. Raising the velocity from 1 to approx. 5 cm/sec. caused a sharp increase in the number of barnacles with their cirri extended. Above 5 cm/sec up to the highest speed obtained in the apparatus (30 cm/sec) there was little or no increase in the number reacting, and in some experiments there were signs that very fast currents had an inhibiting effect.

Sensitivity to water movement was very strong. Extension of the cirri

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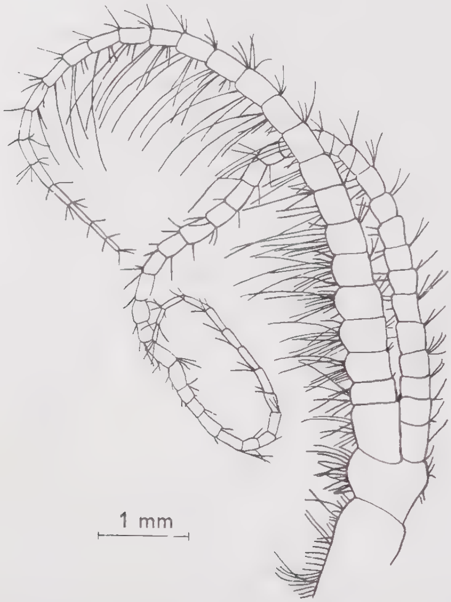
* Identified by Dr R. B. Pike. † Identified by Dr D. Atkins.

TABLE 2. EFFECT OF WATER MOVEMENT ON THE BEHAVIOUR OF *HEXELASMA HIRSUTUM*

	(Water speed cm/sec)	Min from start	No. with cirri extended, out of 47
Expt. 1. In constant temp. room at 5° C; overnight without water movement. Light for Expt. 48 W.	0	0	0
	0·8	10	0
	2·2	12	1
	2·2	17	2
	3·5	18	2
	3·5	23	5
	3·5	24	6
	13	25	6
	13	31	8
	0	31½	2
	0	32	3
	0	34	1
	0	38	3
	0	158	0
Expt. 2. Conditions as Expt. 1	0	5	0
	0·4	10	0
	0·8	15	0
	20	20	3
	17	23	8
	17	25	7
	1·7	30	2
	6	35	7
	3·6	40	5
	5	45	7
	33	45½	8
Expt. 3. Temp. 7° C, other conditions as Expt. 1	0	0	0
	0·6	5	(1 intermittent)
	1·4	10	(2 intermittent, (3 'pumping')
	14	13	9
	4	18	7
	8	23	14
	25	23¼	10
	0	23½	6
	0	24	3
	0	26	1
	0	31	0
Expt 4. Four hours without water movement; temp. 8° C; other conditions as Expt. 1	0	0	(1 intermittent)
	0·4	5	(1 intermittent)
	0·6	10	(1 intermittent)
	1·3	15	1
	3·3	20	3
	17	25	7
Expt. 5. Overnight without water movement at 4° C then temp. reduced to 0·3° C in 2 h using current of 17 cm/sec to circulate water over cooling vessels of ice and salt	0·8	5	(2 intermittent)
	1·2	10	(3 intermittent)
	11	15	4
	17	20	6
	25	25	7
Expt. 6. Specimens kept at 10° C for 2 days, then brought into room with diffuse daylight, temp. 15·6° C.	0	5	(5 intermittent)
	0·7	10	6
	7	15	9
	10	20	6

occurred within a few seconds of starting the paddle wheel that generated the current, and most of the specimens withdrew their cirri a few seconds after the paddle wheel stopped, although one or two remained extended for 5 min or so after prolonged periods of current stimulation. Slowing down the velocity of the current from over 10 cm/sec to less than 5 cm/sec caused an immediate reduction in the number extended; an immediate increase could be obtained by speeding up the current again (Table 2, expts. 2 and 3).

Some of this sensitivity to water currents may lie in the peculiar 3rd cirri of *Hexelasma*. The rami are usually of unequal length, as in most Chthamalidae, with a ratio varying from 1·4:1 down to almost 1:1 (the latter in older specimens). The longer ramus is very flexible, and thinner than the short ramus (Text-fig. 5). When the cirri are extended in a water current (Pl. 1, figs. 2, 3) the long ramus streams out and contrasts with the rest of the cirral net which is held stiffly in the current. The flexible ramus is insensitive to touching that causes bending of some of the other rami, and does not therefore appear to be part of a captorial apparatus. Possibly the long rami function as direction indicators, allowing the cirral net to be swung into the best position for capturing particles carried by the current (Pl. I, figs. 4, 5).



Text-fig. 5. Third cirrus of preserved specimen of *Hexelasma hirsutum*, 9 mm diameter.

In contrast to *Hexelasma*, *Verruca* may extend its cirri in still water. Nevertheless, some experiments showed that water currents of 5–25 cm/sec would stimulate closed specimens of *Verruca* to full extension. Specimens of the holothurian *Psolus squamatus*, which were present on stones with the barnacles, were also observed. Extension of the tentacles was stimulated by water movement, though the extension was very much slower than cirral extension in the barnacles.

Temperature

Experiments on the effect of temperature on extension of the cirri of *Hexelasma* are reported in Table 3. Up to one-third of the specimens under observation would extend their cirri between 7° and 12° C. Outside this optimum range some extension was observed between 0° and 20° C. Some activity, but not full extension, was shown up to 24·5° C., at which temperature more than 50% succumbed to heat coma. *Verruca* also showed heat coma at this temperature. The range of temperatures over which *Hexelasma* would extend the cirri is greater than that reported previously for the 1956 material. The latter showed extension only between 3° and 8° C.

TABLE 3. EFFECT OF TEMPERATURE ON THE BEHAVIOUR OF *HEXELASMA HIRSUTUM*

(Water current approx. 7–25 cm/sec.)

Temperature (° C)	Number of specimens out of 47 showing extension of cirri
−0·4	(10 intermittent)
0	(5 intermittent)
0·1	7
0·3	7
0·8	7
1·6	8
5·0	8
7·0	10–14
8·0	7
9·0	8–13
11·3	8
12·1	8–11
15·6	9
16·5	(5 intermittent)
18·0	8
19·8	3
20·5	(4 intermittent)
22·2	(2 intermittent)
24·0	(1 intermittent)
24·5	50% coma
26·5	100% coma

Both the 1956 and 1957 collections of *Hexelasma* were first examined 5–6 days after capture, but during this period they were treated differently. The 1956 material was brought back on the deck of R.V. 'Sarsia' under a flow of sea water from the deck supply; the specimens must have been subjected to great temperature variation during the voyage and may have suffered damage. The 1957 material was transferred immediately on capture to a refrigerator at 5–7° C and brought back to the laboratory constant temperature room which was also at 5–7° C. Since the sea temperature at the depth the specimens came from was about 7–9° C (see Table 4), there would be much less chance of shock or damage.

Orientation of Hexelasma

The sensitivity of *Hexelasma* to water movement suggested that it might settle or grow in a position with the cirral net facing the prevailing water current in the habitat, as do many other barnacles (Crisp & Stubbings, 1957). Since the stones were very irregular, and as their original orientation on the sea bed was not known, the possibility of orientation to a current was tested by measuring the correspondence of the carino-rostral axes of barnacles on stones bearing groups of five or more. Of fifty-six barnacles measured in this way, the approximate angles subtended between them and a selected reference specimen (usually the largest in the group) were, in percentages:

0° C	45° C	90° C	135° C	180° C
48	30	9	6	7

That is, on average, three out of four specimens in each group faced the same way, or at an angle that allowed them to swing the cirral net, which easily traverses an arc of 90° (Pl. I, figs. 4, 5), to face the same way. It is not known, of course, whether all groups tested faced the same way, but it is a reasonable assumption that they did. The specimens facing away from the direction of the majority were mostly on large or irregular stones and may have been orientated to minor reverse eddies.

DISCUSSION

Barnacle communities of shallow water areas are best developed on wave-beaten rocky coasts or in estuaries with strong tidal currents, and it is a general rule that most species need water movement in their habitat to succeed in competition with other sessile organisms. This rule seems to apply to the deep-water species described here; their behaviour and orientation strongly suggest that they receive considerable water movement in their natural habitat.

These barnacles, and many other common animals in the same habitat, are sessile, and need hard substrata to grow on. Hard substrata are more likely to be exposed where water movement prevents the accumulation of fine particles. At the stations where the barnacles were most abundant the rocks and stones were partially embedded in a muddy bottom, as shown by the absence of a black deposit of manganese oxide present on the exposed parts (see Peach, 1912). Similar partly embedded rocks have been dredged elsewhere, and their position in the deposit, as deduced from the blackening, has been held as evidence that they were once buried in the deposit and more recently exposed by water currents that carried away the fine particles (Peach, 1912).

Biologically and geologically, therefore, there is strong evidence for the existence of water currents where the barnacles occur. The velocity of the currents, judging from the behaviour of the barnacles, must be at least 1–5 cm sec (0·9–4·3 km day); such a current would be able to remove particles of fine sand (0·02–0·2 mm diameter) as well as the finer particles of silt and clay.

SUMMARY

Hexelasma hirsutum (Hoek) and *Verruca recta* Aurivillius are common at many stations on the continental slope to the south-west of the British Isles, the former at depths of 500–1000 fm., the latter from 200 to 1000 fm. Certain external and internal features of these species are described and their systematic status redefined. Their behaviour in the laboratory suggests that they are accustomed to considerable water movement in their natural habitat, and the significance of this inference is discussed.

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APPENDIX

Environmental conditions on the slope

By L. H. N. Cooper

In Table 4 an attempt is made to assess the hydrographic conditions under which the larger barnacle, *Hexelasma hirsutum*, was living. It will be seen that temperatures ranged from 4·2° to 9·4° C., salinities were between 35·1 and 35·5‰, and the water was well oxygenated. These temperatures agree with the range of temperatures over which *Hexelasma* showed most activity.

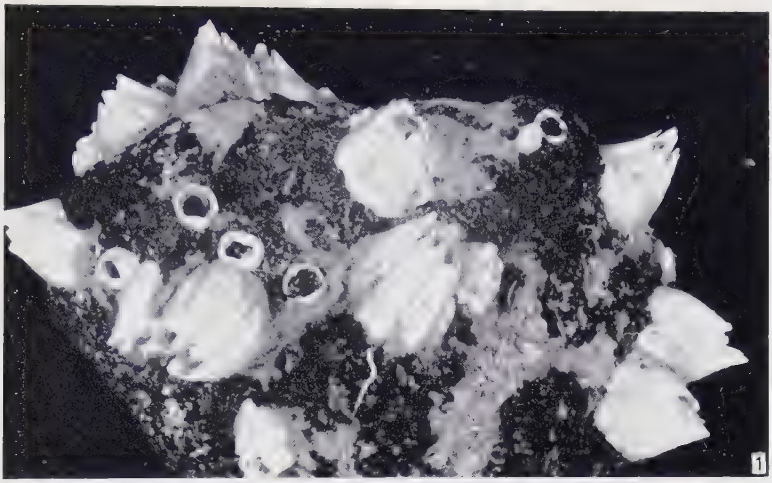
TABLE 4. HYDROGRAPHIC CONDITIONS AT STATIONS ADJACENT TO THOSE AT WHICH LIVING *HEXELASMA HIRSUTUM* WERE TAKEN

Dredge station	Depth (fm.)	Temp. (° C)	Salinity (‰)	Oxygen (ml./l.)	Silicate (μg-atom/l.)
48° 31′ N., 10° 03′ W.	690	6·9–7·7	35·4	4·5	13
59° 40′ N., 7° 21′ W.	516	8·4	35·3	—	—*
38° 31′ N., 26° 49′ W.	845	7·9–8·7	35·3	—	—†
48° 04′ N., 9° 23′ W.	1000	4·2	35·0	5·0	20
48° 02′ N., 9° 25′ W.	930	4·5	35·1	5·1	15
48° 02′ N., 9° 27′ W.	900	4·6	35·1	5·2	15
48° 33′ N., 10° 05′ W.	570–770	6·5–9·4	35·3–·5	4·1– ·8	12–14
48° 34′ N., 10° 00′ W.	570–700	6·7–9·4	35·3–·5	4·2–5·8	11–14

* From Tait (1957). † From International Council (1944, p. 126); the remainder from unpublished data, L. H. N. Cooper.

It is probably coincidental, but water at all stations would have contained a proportion of 'Gibraltar Water' formed by mixing of warm, deep, saline water from the Mediterranean basin with deeper North Atlantic Central water. The occurrence of *Hexelasma* at the Azores suggests that the barnacle is not in need of materials which may be carried by cascading water from the continental shelf.

To the physical oceanographer the inference that the habitat of these deep-water barnacles is one of considerable water movement is of much importance. Such water movements could be oscillating surges resulting from internal waves or steady currents running along the slope, and it would be helpful if the barnacles themselves could be orientated. Dried specimens of *Hexelasma* were sent to Dr R. S. M. Nairn of Newcastle, in the hope that they might have measurable magnetic orientation, when the direction of the water current could be assumed to be the same as the carino-rostral axis (i.e. the direction which the cirral net faces). However, no deflexion of the magnetometer needle was observed. Sometimes iron objects discarded from ships are dredged up: if such an object is found in future, with barnacles growing on it, the residual magnetism may provide a measure of the direction of water movement *in situ*.



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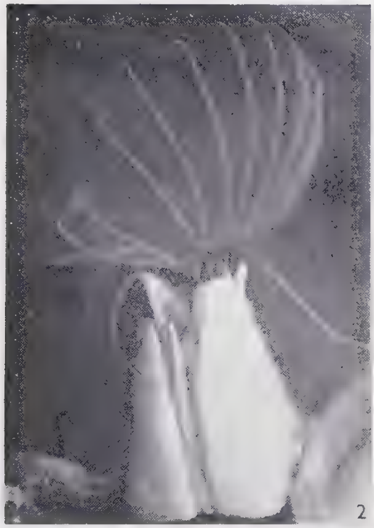

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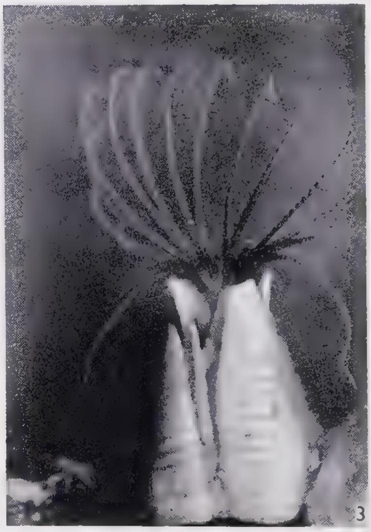

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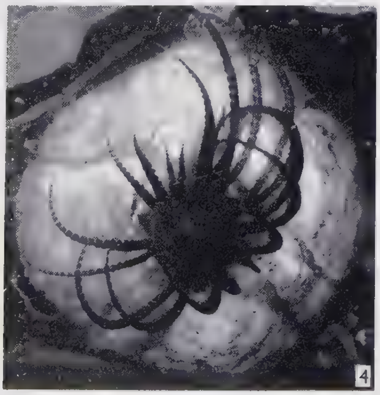

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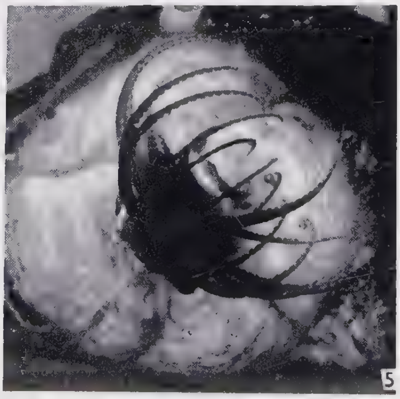

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EXPLANATION OF PLATE I

Fig. 1. Group of *Hexelasma hirsutum* on a piece of rock, which also bears one live and several dead specimens of *Verruca recta*, as well as patches of sponge and a serpulid tube. × ½.
Fig. 2. *Hexelasma* viewed from the carinal aspect, showing cirral net turned to face water current coming from the left. Note long rami of 3rd cirri streaming in current. × 1½.
Fig. 3. The same specimen as fig. 2 just after water current was stopped.
Figs. 4, 5. View of larger *Hexelasma* from above showing rotation of cirral net. Current from left. In this specimen the 3rd cirri have almost equal rami. Approx. natural size.

Figs. 2–5 taken at 5° C in constant temperature room, with specimens subjected to water current of 5–20 cm/sec in apparatus described by Southward (1957). Approx. 2:1 on negative (Pan F); electronic flash tube ½–1 ft. away, 100–300 J; Elmar 50 mm lens at f18.

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THE BREEDING SEASON OF SOME LITTORAL ASCIDIANS IN SCOTTISH WATERS

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(Text-fig. 1)

In previous papers (Millar, 1952; 1954*a*; 1954*b*) I have dealt with the reproductive cycle of several ascidians in Scottish waters, and in the present account observations are extended to other species.

The course of breeding was studied in the following species. Family Polyclinidae: *Sidnyum turbinatum* Savigny, *Aplidium nordmanni* (Milne Edwards), *A. punctum* (Giard), *A. pallidum* (Verrill) and *Polyclinum aurantium* Milne Edwards. Family Didemnidae: *Didemnum candidum* Savigny and *Lissoclinum argyllense* Millar. Family Clavelinidae: *Clavelina lepadiformis* (Müller).

Material was collected at intervals, mostly of 1–2 months, throughout the years 1952 and 1953, from the lower part of the shore of the island of Luing, Argyll. At the collecting station Luing and a neighbouring island converge to form a narrow sheltered channel much of which dries at low water of spring tides, and in this area ascidians of several species are abundant.

Breeding condition of each sample was measured by the percentage of specimens having embryos or larvae in the zooids (Families Polyclinidae and Clavelinidae) or in the common test (Family Didemnidae).

Fig. 1 (C–H, J, K) shows the course of breeding in the eight species just studied. Comparable results for other species previously investigated (Millar, 1952; 1954*a*; 1954*b*) are also shown graphically (A, B, I, L). All results are plotted on the same time base, although they refer to different years, this procedure being justified, first by the need to get a general picture of breeding, and second by the relatively small year-to-year variations.

A relationship between sea temperature, breeding and geographical distribution in marine animals has been widely recognized, particularly by Appellöf (1912), Orton (1920), Runnström (1927, 1929, 1936), and Hutchins (1947). Orton noted that temperature apparently controls the breeding season in many marine animals, and Runnström found experimentally that embryonic development of species occupying the high, middle and low latitudes of the boreal region was possible only at the temperatures prevailing in the corresponding geographical ranges.

The ascidian species of which the breeding period in Scottish waters is now known fall into three geographical groups, as follows: (1) boreo-arctic

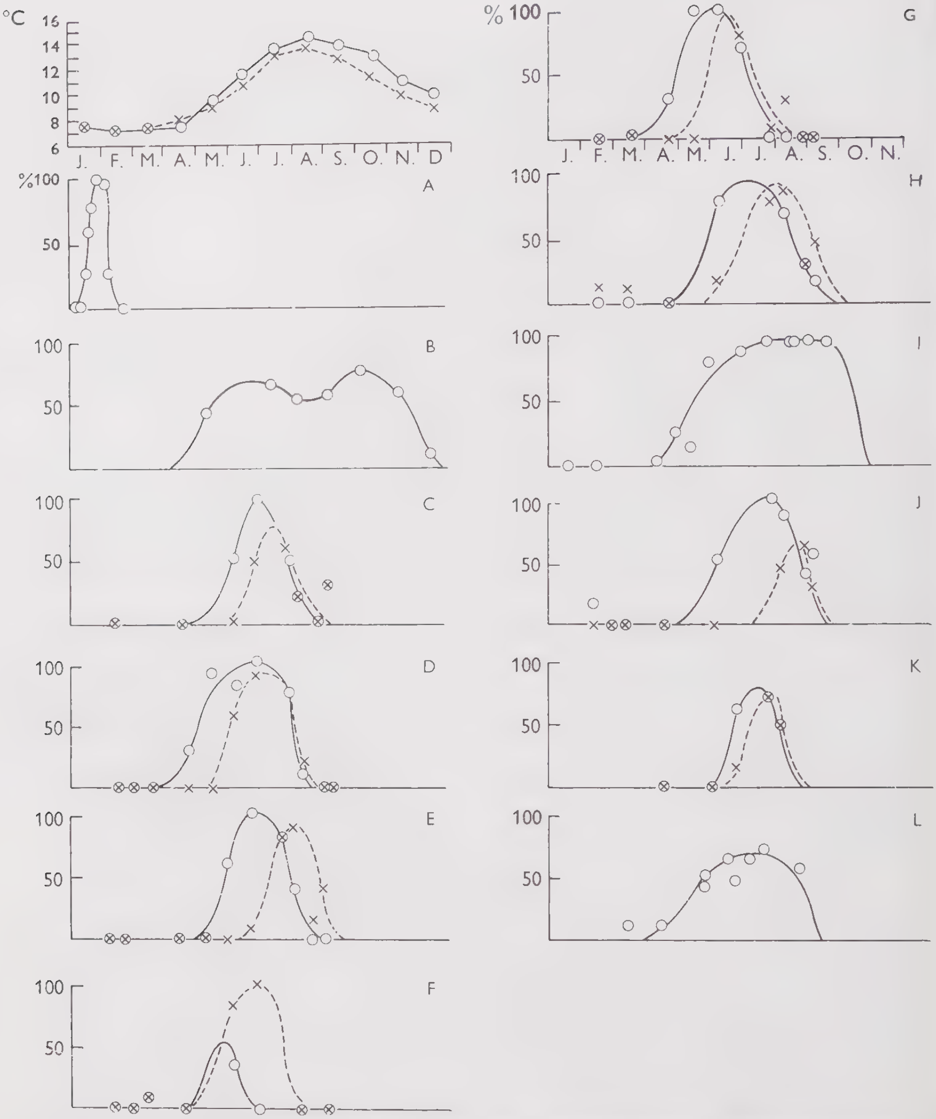

Fig. 1. Smoothed curves showing the course of breeding as represented by the percentage of each sample with developing embryos (○—○) and with larvae (×---×) in the colonial species (C–L). In *Pelonaia corrugata* (A) the curve shows the percentage of each sample in the process of spawning, as indicated by the condition of the gonad. In *Dendrodoa grossularia* (B) the curve shows the percentage of each sample with developing embryos or larvae. At the top left corner of the figure is shown the monthly mean sea surface temperature at Millport for the year 1952 (×---×) and 1953 (○—○). A, *Pelonaia corrugata;* B, *Dendrodoa grossularia;* C, *Aplidium pallidum;* D, *Sidnyum turbinatum;* E, *Aplidium nordmanni;* F, *A. punctum;* G, *Polyclinum aurantium;* H, *Didemnum candidum;* I, *Diplosoma listerianum;* J, *Lissoclinum argyllense;* K, *Clavelina lepadiformis;* L, *Botryllus schlosseri.*

species centred in arctic and extending southwards into boreal waters, e.g. *Pelonaia corrugata* Forbes and Goodsir; (2) boreo-arctic species centred in boreal and extending northwards into arctic waters, e.g. *Dendrodoa grossularia* (van Beneden) and ? *Aplidium pallidum* (Verrill); (3) true boreal species, including *Sidnyum turbinatum* Savigny, *Aplidium nordmanni* (Milne Edwards), *A. punctum* (Giard), *Polyclinum aurantium* Milne Edwards, *Didemnum candidum* Savigny, *Diplosoma listerianum* (Milne Edwards) and ? *Lissoclinum argyllense* Millar.

The question mark before *Aplidium pallidium* indicates that its centre of distribution is uncertain, and the one before *Lissoclinum argyllense* that records are still very scarce. The remaining six of the eight species newly studied are boreal.

It is evident from Fig. 1 that the breeding periods are of three kinds: (1) short and restricted to the winter (A), (2) long, and extending from spring to late autumn (B), (3) short or moderately long, and restricted to the summer (C–L).

These three groups of species, defined by the time and extent of the breeding season, are the same groups as those defined earlier by geographical distribution. It appears, therefore, that in the species studied the time and extent of the breeding period is related to the centre of geographical distribution of the species. Water temperature is the factor most likely to control the breeding period.

North boreo-arctic species are in British waters near their southern limit, and the short breeding period of *Pelonaia corrugata* suggests that the southern boundary of species in this group may be fixed by winter temperatures too high for breeding farther south.

Boreal, and especially south boreal, species are in British waters near their northern limit and the short breeding period of several boreal ascidians suggests that the northern boundary is probably fixed by summer temperatures too low for breeding, farther north.

SUMMARY

The breeding season is given of a number of ascidian species on the Scottish west coast.

The species can be divided into north boreo-arctic, south boreo-arctic, and boreal species, and each of these groups has a characteristic duration and time of breeding season.

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THE BREEDING OF THE SCALLOP, *PECTEN MAXIMUS* (L.), IN MANX WATERS

By James Mason
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(Plates I and II and Text-figs. 1–5)

MATERIAL AND METHODS

An important fishery for the scallop, *Pecten maximus* (L.), is carried on during the winter months round the Isle of Man. A knowledge of the breeding of this lamellibranch would be useful should legislation become necessary with regard to the fishery.

Regular samples of scallops were dredged, whenever possible, at roughly weekly or fortnightly intervals throughout the period October 1950–October 1952, from a depth of 13–16 fm. off Bradda Head and Bay Fine, near Port Erin. Approximately 8000 scallops of all ages up to 13 years were examined, though very few young ones were caught. Scallops were aged by means of the growth-rings on the shell, of which one is laid down each spring (Mason, 1957).

The gonad of every scallop was examined macroscopically and classified as regards degree of development (see p. 656). *Pecten maximus* is a hermaphrodite, the gonad consisting of separate male and female parts.

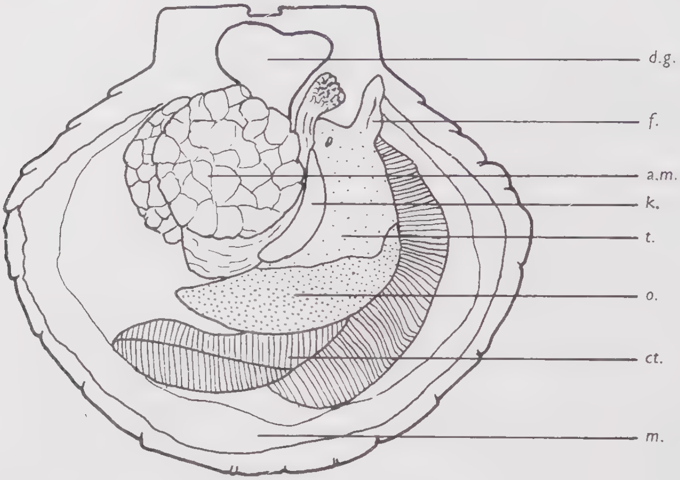
Several gonads in each stage of development were examined histologically by means of transverse sections, and serial sections were prepared of the whole of some small gonads. The material was always taken so as to include both ovarian and spermatic tissue. Three fixatives were used, Bouin, Heidenhain's Susa, and Zenker, of which Bouin proved the most satisfactory.

There is always some loss of reproductive cells from the cut surface of the gonad, and so all pieces to be fixed were cut large enough to allow for this loss. Penetration of the fixative was facilitated by making needle-holes in the material, which was then placed in the fixative and left for several days. The material was then cut into halves, each half containing both ovary and testis, and replaced in the fixative. This second cutting involved no loss of reproductive cells. After dehydration with ethyl and *n*-butyl alcohols, clearing in cedarwood oil and embedding in paraffin wax, sections were cut parallel to the newly-cut face. Sections were cut between 5 and 8 μ thick, and stained with Ehrlich's acid haematoxylin and eosin.

Fixation always resulted in a slight contraction of the gonad, and so did handling.

THE STRUCTURE OF THE GONAD

The mature gonad of *Pecten maximus* was described, but not in detail, by Dakin (1909). The single gonad is posterior and ventral to the rudimentary foot, forming a tongue-shaped mass attached to the adductor muscle (Text-fig. 1). The proximal part is white and forms the testis; the ovary is orange-red and lies distal to the testis. A loop of the alimentary canal passes through the gonad, penetrating into the ovary. This loop cannot be seen in the mature gonad unless, as occasionally happens, it passes close to the free, ventral edge of the ovary.



Text-fig. 1. The gonad of *Pecten maximus in situ*, seen from the right side. *a.m.*, adductor muscle; *ct.*, ctenidium; *d.g.*, digestive gland; *f.*, foot; *k.*, kidney; *m.*, mantle; *o.*, ovary; *t.*, testis.

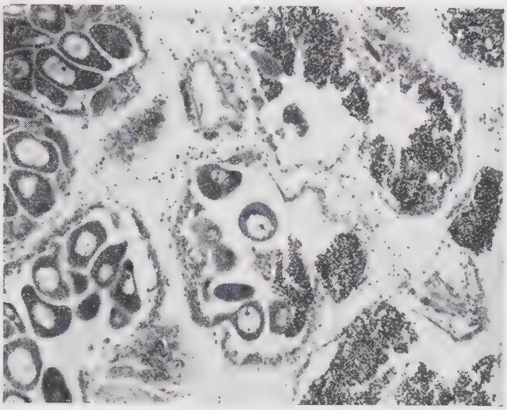
The boundary between testis and ovary is usually quite sharp, though irregular, but sometimes islets of tissue of one kind occur within the tissue of the other kind. This irregularity in the distribution of the spermatic and ovarian tissue occasionally reaches a state in which the gonad is almost exclusively either male or female. I found two gonads composed entirely of female tissue.

The gonad is made up of many branched, ciliated tubules or ducts, which bear many sacs, the alveoli or follicles. The ducts are round in cross-section, 70 μ or more in diameter. The lumen may be as small as 20–25 μ, and is lined with ciliated epithelium. In serial sections the ducts may be traced and are seen to join and rejoin. They eventually join one of the two main ducts, one on each side, which are much wider, up to 1000 μ in section, and open dorsally, one into each kidney.

The sexual products arise by proliferation of the germinal cells which line the follicle walls. The follicle wall is usually less than 1 μ thick. A few scattered nuclei, 4–7 μ long, which are elongated and sometimes spindle-shaped, may be seen in the follicle wall, though they may be difficult to find in a full gonad.

The follicles become filled with sexual products, and those near the surface of the gonad appear to the naked eye as small, rounded, red or white bodies; the full follicles range in size from 300 to 700 μ.

In each follicle all the contents are normally of one sex, though in one gonad follicles were found containing both male and female elements, spermatozoa, spermatocytes and large oocytes being found side by side (Text-fig. 2).



Text-fig. 2. Transverse section of an abnormal gonad, showing ambisexual follicles. × 100.

The male follicle usually contains a few early stages of spermatogenesis near the follicle wall, but the lumen is filled with spermatozoa. Each spermatozoon has a minute conical head, about 1·4 μ, which stains intensely in haematoxylin, and a tail about 50 μ long. The spermatozoa are arranged radially from the centre of the follicle, or from the point where the follicle opens into the ciliated duct, with their tails pointing towards the centre or towards the duct. The female follicle contains a few young oocytes attached to the wall, while the lumen is full of large oocytes. The large oocytes appear polygonal in sections, and are packed tightly in the follicles; their greatest diameter in sections is about 80–90 μ. The large oval, or spherical, vesicular nucleus has a diameter roughly two-thirds that of the cell. A delicate network of chromatin fibres extends through the nucleus, and one conspicuous acentrally-placed nucleolus is present. The nucleus is surrounded by granular cytoplasm, which often contains bodies which stain purple in haematoxylin. The whole cell is surrounded by a membrane some 1·5 μ thick, which is not stained by either haematoxylin or eosin, but which stains blue in Azan.

Little connective tissue is present between the follicles of the full gonad, although some is present round the loop of the alimentary canal and the ducts, and sometimes near the outer wall of the gonad. The connective tissue consists mainly of a network of fibres in which are seen various nuclei, spindle-shaped, oval and spherical, whose functional relationships are not known. Among the fibres are a few vacuolated cells. Also present are some small cells, 6–7 μ in diameter, each with a nucleus 2 μ in diameter, surrounded by a clear cytoplasm which stains pink in eosin; similar cells are seen in the lumina of the thick-walled blood vessels which are sometimes seen in sections of the gonad. Also between the follicles are transverse muscle fibres.

The outer wall of the gonad consists of two layers, an outer epithelial and an inner muscular layer. The muscles of the wall, together with the transverse muscles, probably assist the ciliated lining of the ducts in ejecting the genital products.

THE BREEDING OF THE SCALLOP

THE BREEDING CYCLE

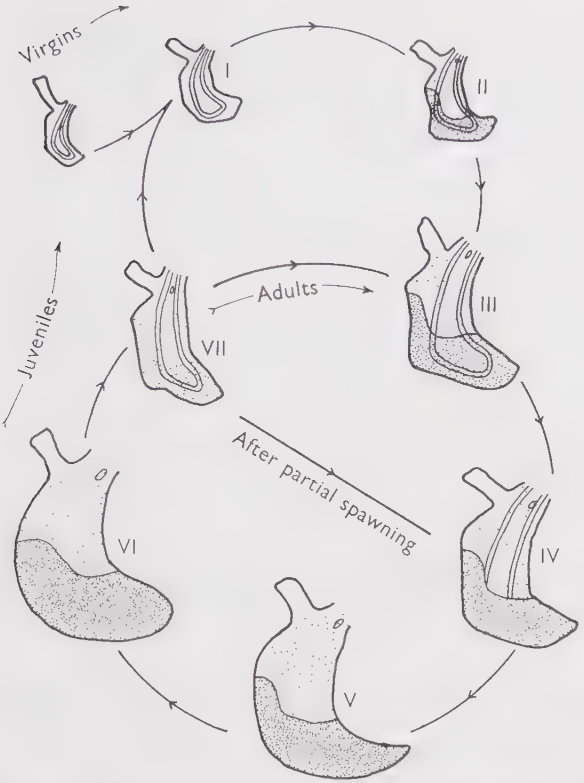
In Manx waters, scallops first spawn in the autumn following the deposition of the second growth-ring on the shell, when most of them are just 2 years old (Mason, 1957). In the following year they have one main spawning, in autumn, and thereafter they have two main spawnings each year, one in spring and one in autumn. In this paper scallops which have never spawned are called virgins, between the first and second spawnings they are called juveniles, and after the second spawning adults.

In order to determine the breeding cycle, gonads from the regular samples of scallops were classified into eight arbitrary stages of maturity, stages 0–VII, which are described in Table 1, the colour nomenclature being that of Ridgway (1912). The external features of the various gonad stages are shown in Text-fig. 3, and their microscopic structure in Text-fig. 4 and Pls. I and II.

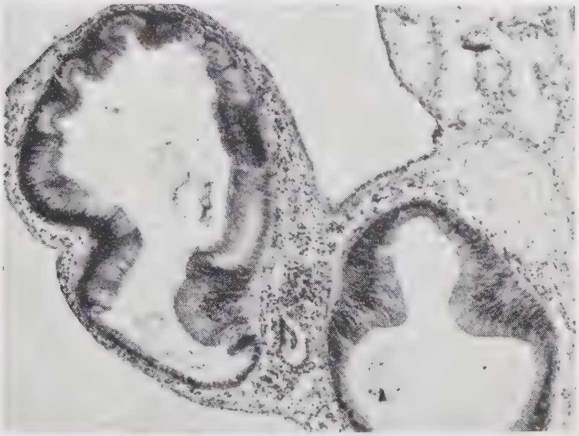
Tang (1941) and Gibson (1956), who made similar studies of scallops, used a classification of only five stages, but their material consisted largely of commercial samples of adult scallops, and no account was taken of the differences between virgins, juveniles and adults. The classification shown in Table 1 assigns three stages (0, I and II) to young scallops, virgins and juveniles, which were not, therefore, represented in the material studied by Tang and Gibson. The 'spawning' stage of Tang and Gibson was not used in the present study, since the duration of the act of spawning was found to be so short as to make it most unlikely that many scallops in the spawning condition would be observed in nature.

The virgin scallop

Table 2 shows the monthly percentages of gonads of virgin scallops in each stage of maturity during the first 2 years or so of the scallop's life. So few young scallops were caught that data for corresponding months in the period considered (e.g. March 1951 and March 1952) were combined, and all the scallops were treated as if they were spawned in the same year.


Virgins
I
II
III
IV
V
VI
VII
Adults
Juveniles
After partial spawning

Text-fig. 3. Macroscopic changes in the gonad of *Pecten maximus*.



Text-fig. 4. Transverse section of stage 0 gonad, 2–3 months old (December). × 100.

TABLE 1. *PECTEN MAXIMUS*. MACROSCOPIC AND

Gonad stage	External features (Text-fig. 3)
0. *Immature (virgin)*	Gonad small, flat and angular, transparent and colourless. No reproductive tissue visible to naked eye. Whole of loop of alimentary canal clearly seen
I. *Developing (virgin) or spent-recovering (juvenile)*	Gonad growing, still flat and angular. Reproductive tissue now visible to naked eye as minute follicles, translucent and sparse. Gonad uniformly fawn-coloured, with no visible differentiation into testis and ovary. Alimentary canal visible
II. *Differentiated gonad (virgin and juvenile)*	Gonad growing, still flat and angular, now obviously differentiated into testis and ovary; male follicles white and female fawn or light salmon orange, colour imparted by contents. Follicles still small and sparse, and alimentary canal still visible
III. *Recovering*	Gonad larger and proportionately thicker, angular. Flabby, containing free water, especially in adults after spawning. Assuming brighter colour, testis white and ovary bittersweet orange. Follicles larger and denser, but still spaces between them, and alimentary canal still visible
IV. *Filling*	Gonad still larger and thicker (thickness about $\frac{1}{3}$ width); still somewhat flabby, containing a little free water. Outline less angular, but not completely smooth. Colouring brighter due to denser colouring of follicles, testis white, ovary bittersweet orange or grenadine pink. Follicles larger and closer together, the latter especially in ovary. Alimentary canal still visible between follicles in testis, but not in ovary, but its outline still discernible owing to thinness of gonad
V. *Half-full*	Gonad again larger and thicker, firmer, and containing very little free water. Rounded, with tapering tip. Brighter, testis creamy-white, ovary grenadine pink or grenadine. Follicles larger, becoming packed together. Loop of alimentary canal invisible, but still causes wall of gonad to bulge
VI. *Full*	Gonad is now at its largest, thickest (thickness about $\frac{1}{2}$ width) and firmest, containing no free water. Rounded to tip. Bright, with follicles highly coloured and closely packed; testis cream coloured, ovary usually grenadine. Loop of alimentary canal indiscernible
VII. *Spent and partially spent*	Spawning may be partial or complete. Gonad dull, angular, thin and collapsed; flabby, containing much free water *Spent gonad* fawn-coloured and loses visible differentiation into testis and ovary after spawning for first time. Older scallops usually retain differentiation, testis yellowish-brown, ovary dull orange pink. Follicles appear empty *Partially spent gonad* always retains differentiation; testis yellowish white, ovary dull, bittersweet orange or orange chrome. Follicles appear hollow, with a coloured ring round periphery indicating retention of some genital products

MICROSCOPIC CHANGES IN THE GONAD

Histological details (Text-fig. 4; Pls. I and II)

Youngest scallops caught (2–3 months old) showed beginning of development, which then continues. Gonad at first almost completely occupied by loop of alimentary canal, but later connective tissue develops. Narrow tubules, bearing primary germ cells on walls, ramify and give rise to follicles and ciliated ducts, rounded in section. Primary germ cells irregular, 8–14 μ, with oval, vesicular nucleus and scattered chromatin, lightly stained in haematoxylin. Follicular cells also present. Primary germ cells give rise to gonia, each with a spherical, vesicular nucleus, 3·5–7 μ; chromatin scattered, but stains more densely than primary germ cell; well-defined nucleolus; usually a little cytoplasm. Formation of gametocytes, shown by synapsis, occurs first in distal (female) part of gonad (synapsis shows as clumping together of chromatin into an irregular, densely-staining mass). Gonad increases in size with formation of follicles and connective tissue. (Text-fig. 4; Pl. IA)

Developing (*virgin*). Follicles growing. Synapsis now occurs in proximal (male) part of gonad. Male follicles lined by several layers of spermatogonia, and lumina filled with synaptic and post-synaptic spermatocytes, with occasionally a few spermatids. Spermatocyte has a little cytoplasm, and appears polygonal in sections; nucleus roughly spherical 2·5–3·5 μ, unevenly distributed chromatin, fairly densely stained, no visible nucleolus. Spermatid 2 μ, spherical nucleus, chromatin evenly or somewhat unevenly distributed. Female follicles have oogonia and synaptic oocytes near walls, and young oocytes up to 30 μ, growing rapidly, in lumina. Young oocytes have somewhat fibrillar cytoplasm; nucleus spherical, vesicular, with a delicate chromatin network and spherical nucleolus. Spaces in female follicles. Much connective tissue between follicles. (Pl. IB, C)

Spent-recovering (*juvenile*) is similar, but has larger spaces in follicles. Before recovery becomes obvious to naked eye many gonia are produced from residual gonia and primary germ cells, and synapsis occurs as above

Spermatozoa appear at centre of male follicles; many synaptic and post-synaptic spermatocytes and some spermatids; several layers of spermatogonia near walls. A few oogonia and synaptic oocytes on wall of female follicles; many half-grown (30–60 μ) oocytes, appearing stalked, with granular cytoplasm, and a few young oocytes. Less connective tissue, but still continuous between follicles. (Pl. ID)

Male follicles contain more spermatozoa, not yet closely packed, arranged radially; still many spermatogonia and spermatocytes near walls, and some spermatids. Few oogonia and synaptic oocytes in female follicles, and lumina almost filled with growing oocytes, mostly half-grown, a few larger and smaller. Few spaces left in follicles, rather more in adults (in which stage III is the first recognizable stage of recovery after spawning) than in virgins and juveniles. Connective tissue still present, but disappearing. Main genital ducts becoming flattened. (Pl. IE)

Spermatogonia still form a continuous layer on walls of male follicles, and inside them a band of spermatocytes and a few spermatids; lumina contain many spermatozoa arranged radially. Few oogonia and synaptic oocytes in female follicle; fewer young oocytes; lumen contains more half-grown and a few almost fully grown (60–80 μ) oocytes. Little connective tissue except round alimentary canal and ducts. Main ducts larger and more flattened. (Pl. IF, G)

A few spermatogonia and spermatocytes remain near walls of male follicles, lumina full of spermatozoa which are becoming closely packed. Walls of female follicle lined with a few young and half-grown oocytes; lumina filled with almost fully grown oocytes, each with germinal vesicle still intact; very few oogonia and synaptic oocytes, indicating that production of oocytes has now almost ceased. Very little connective tissue

Follicles maximum size. Male follicles packed with spermatozoa, still arranged radially; few scattered spermatogonia and spermatocytes persist near walls. Female follicles packed with fully-grown (80–90 μ) polygonal or pear-shaped oocytes, whose germinal vesicles show signs of breaking down; very few scattered oogonia and young oocytes persist near walls. No connective tissue except round alimentary canal and ducts. Main ducts flattened. (Pl. IIH, J)

Follicles smaller and collapsed, containing large spaces. Main ducts wide. Some connective tissue visible

Spent gonad after spawning for first time contains a few residual primary germ cells, spermatogonia and spermatocytes on walls of male follicles, but few or no spermatozoa; female follicles have a few primary germ cells, oogonia and young oocytes on walls. Older scallops retain more spermatocytes and a few spermatozoa, and more young and some half-grown oocytes. (Pl. IIN)

Partially spent gonad retains more genital products. Many residual spermatocytes and spermatozoa, and half-grown and almost fully grown oocytes. (Pl. IIM)

TABLE 2. MONTHLY PERCENTAGES OF VIRGIN SCALLOPS AT EACH STAGE OF MATURITY DURING THE FIRST 2–2½ YEARS OF LIFE

[Owing to the small numbers caught, data for scallops caught in corresponding months of the period of the investigation (e.g. March, 1951 and March, 1952) are combined, and all the scallops are treated as if they were spawned in the same year.]

	Gonad stages								
Month	0	I	II	III	IV	V	VI	VII	No. of gonads
Apr.	—	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—	—
June	—	—	—	—	—	—	—	—	—
July	—	—	—	—	—	—	—	—	—
Aug.	—	—	—	—	—	—	—	—	—
Sept.	—	—	—	—	—	—	—	—	—
Oct.	100·0	—	—	—	—	—	—	—	2*
Nov.	—	—	—	—	—	—	—	—	—
Dec.	100·0	—	—	—	—	—	—	—	1
Jan.	100·0	—	—	—	—	—	—	—	2
Feb.	100·0	—	—	—	—	—	—	—	12
Mar.	100·0	—	—	—	—	—	—	—	17
Apr.	100·0	—	—	—	—	—	—	—	3†
May	100·0	—	—	—	—	—	—	—	9†
June	100·0	—	—	—	—	—	—	—	5
July	100·0	—	—	—	—	—	—	—	2
Aug.	100·0	—	—	—	—	—	—	—	5
Sept.	100·0	—	—	—	—	—	—	—	6
Oct.	100·0	—	—	—	—	—	—	—	12
Nov.	100·0	—	—	—	—	—	—	—	23
Dec.	100·0	—	—	—	—	—	—	—	18
Jan.	100·0	—	—	—	—	—	—	—	15
Feb.	97·7	1·2	1·2	—	—	—	—	—	85
Mar.	92·3	5·5	2·2	—	—	—	—	—	91
Apr.	40·2	27·8	8·2	12·4	11·3	—	—	—	97‡
May	22·7	14·7	28·0	20·0	10·7	4·0	—	—	75‡
June	10·2	28·6	16·3	10·2	16·3	6·1	12·2	—	49
July	—	17·4	21·7	17·4	30·4	8·7	4·3	—	23
Aug.	—	8·3	13·9	8·3	8·3	5·6	5·6	50·0	36
Sept.	—	—	—	8·1	13·5	5·4	9·5	63·5	37

* Millport scallops. † 1st growth ring. ‡ 2nd growth ring.

EXPLANATION OF PLATES I AND II

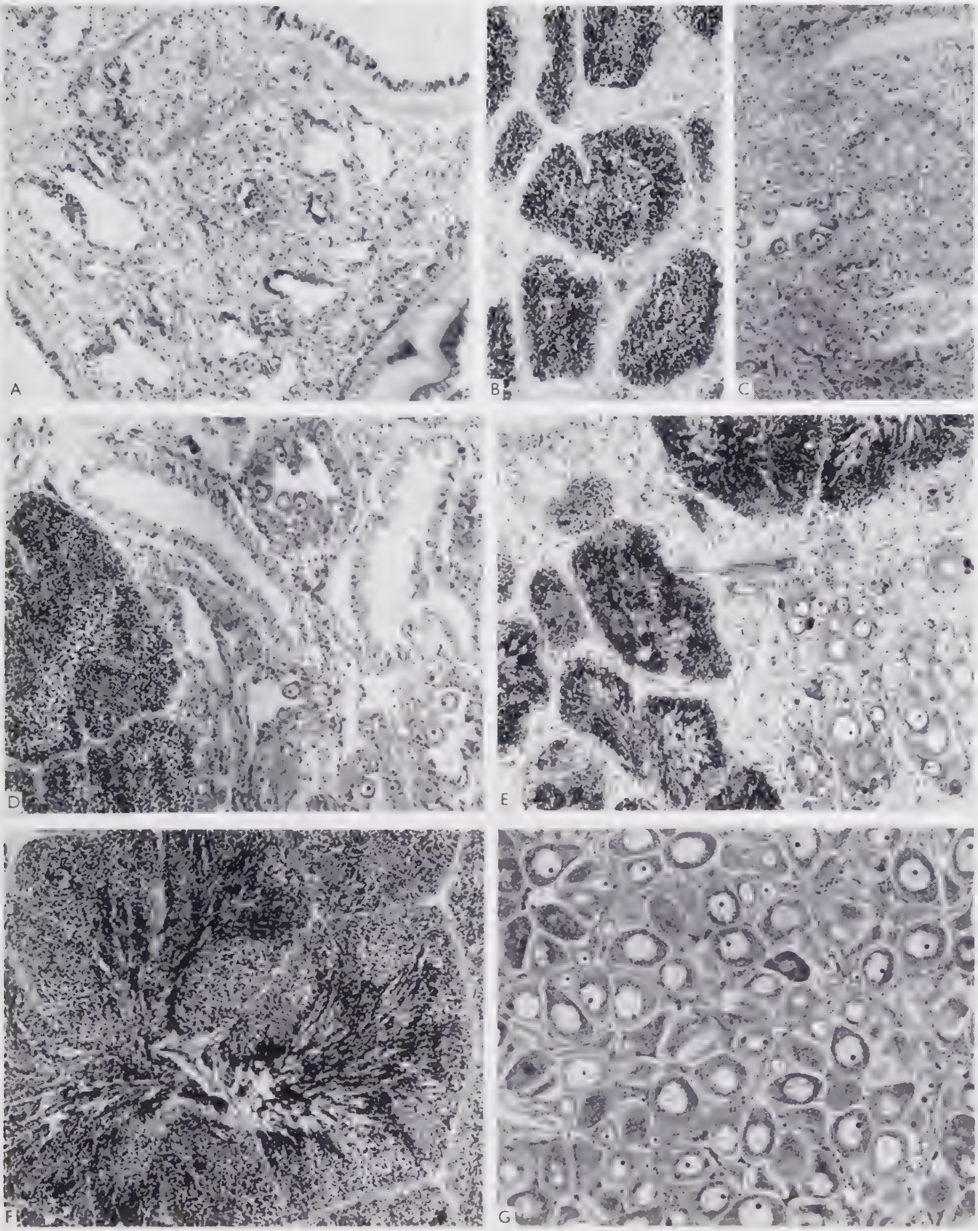
Pecten maximus. Photomicrographs of transverse sections through gonads in the various stages of maturity.

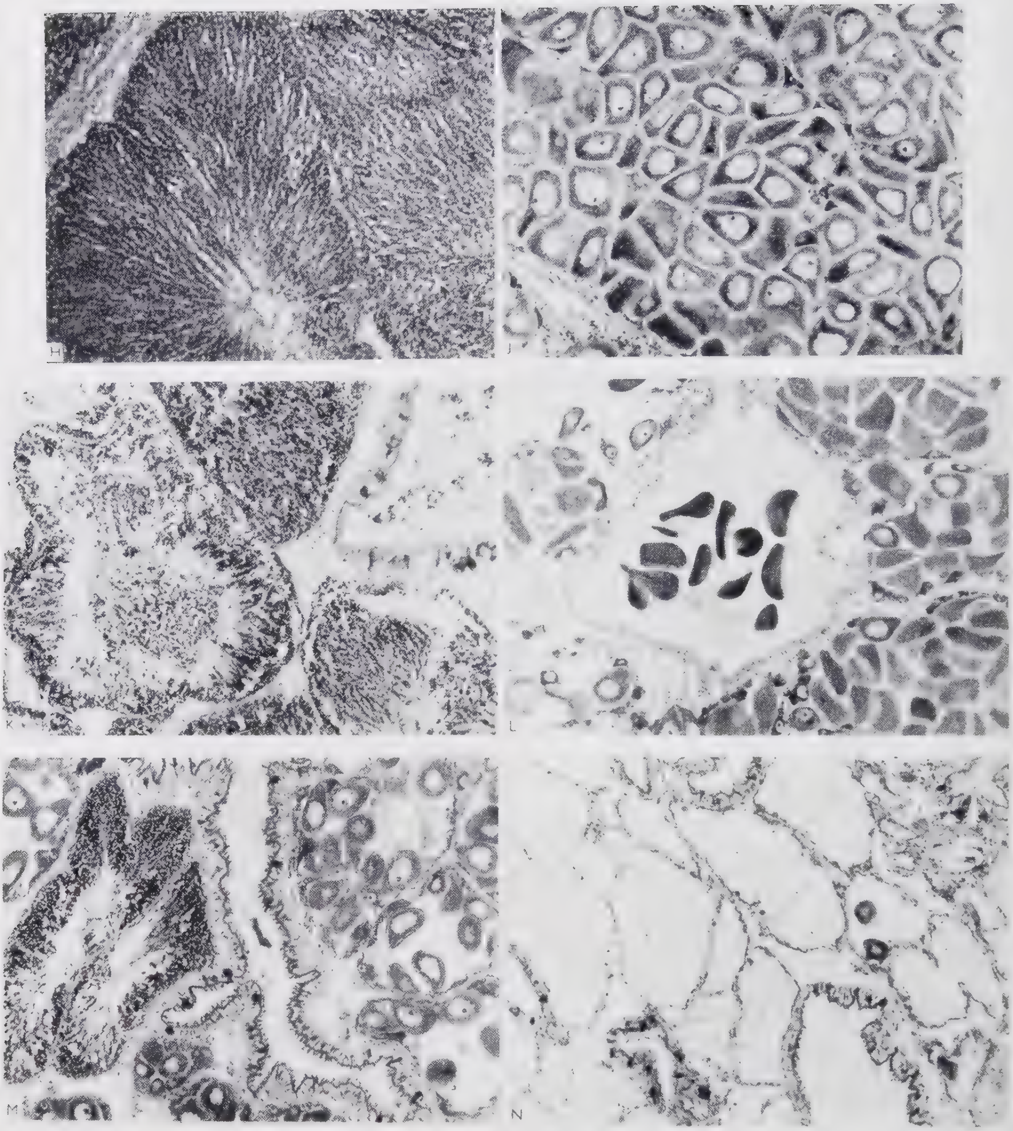
Plate I

A. Stage 0 gonad, at time of deposition of second growth ring (April). Gonia showing as thickenings on edge of follicles. ×96.
B. Stage I testis. ×96.
C. Stage I ovary (cells with enlarged nuclei are young oocytes). ×96.
D. Stage II testis and ovary. ×96.
E. Stage III testis and ovary (spermatozoa have appeared in the former). ×96.
F. Stage IV testis. ×96.
G. Stage IV ovary. ×96.

Plate II

H. Stage VI testis. ×93.
J. Stage VI ovary, showing fully-grown oocytes. ×93.
K. Running testis, showing sperm in genital ducts. ×93.
L. Running ovary, showing mature ova. ×93.
M. Stage VII testis and ovary (partially spent). ×93.
N. Stage VII testis and ovary (spent). ×93.


A
B
C
D
E
F
G


H
J
K
L
M
N

All scallops with no growth-rings and almost all with one ring possessed stage 0 (immature) gonads, and the first sign of development obvious to the naked eye appeared in the late winter or early spring about the time of deposition of the second growth-ring, although development of tubules and germ cells had been going on continuously since a very early age (Table 1). Development continued throughout the spring and summer, through stages I to VI, and culminated in the act of spawning in the August or September after the deposition of the second growth-ring. Spawning was complete in most individuals, and involved the complete loss of differentiation into testis and ovary. The macroscopic and histological details of the various stages of gonad development are given in full in Table 1.

The juvenile scallop

The breeding cycle of the juvenile scallop begins with those scallops which have just spawned for the first time in August or September. Since the juveniles themselves spawn in the following August or September, the period of the investigation, October 1950–October 1952, almost covers two complete breeding cycles.

The gonads began to show signs of recovery externally in October or November (Table 3), although production of gonia had occurred before this. Since most gonads had spawned completely and lost their differentiation into testis and ovary, it was possible to distinguish stages I (spent-recovering) and II (differentiated), which cannot be distinguished in adult gonads owing to their retention of more genital products, and, therefore, of their differentiation. Recovery continued throughout the winter, stages III and IV predominating from January to March. The third growth-ring was laid down on the shell in the spring. Stage V gonads were commonest in April and May, and by June or July most juvenile scallops had full (stage VI) gonads. The main spawning occurred in the second half of August in 1951 and between 5 and 11 September in 1952 (Table 3B), and most scallops of this age took part. Spawning was almost complete in most individuals, but more sexual products were retained than in the first (virgin) spawning, and the gonads usually retained their differentiation. In addition, some juvenile scallops released a very small proportion of their gametes between 9 and 17 July 1952 (Table 3B).

The adult scallop

The principal spawning of adult scallops, like that of juveniles, was found to occur in August or September, so that two annual breeding cycles are again almost covered by the period October 1950–October 1952. At the beginning of each cycle were found mostly spent and a few partially spent gonads resulting from the spawning of juvenile and adult scallops (Table 4). Recovery soon began, and, owing to the larger numbers of reproductive cells

TABLE 3. PERCENTAGE OF JUVENILE SCALLOPS AT EACH STAGE OF MATURITY

(A) MONTHLY PERCENTAGE

(All samples in each month combined.)

October 1950–September 1951

	Gonad stages							
Month	I	II	III	IV	V	VI	VII	No. of gonads
Oct.	—	—	—	—	—	—	100·0	6
Nov.	25·0	8·3	16·7	8·3	—	—	41·7	12
Dec.	—	25·0	—	50·0	—	—	25·0	4
Jan.	17·6	19·6	5·9	33·3	7·8	—	15·7	51
Feb.	17·0	5·4	19·7	39·5	4·8	—	13·6	147
Mar.	11·9	6·8	13·7	36·3	15·9	7·9	7·9	278
Apr.	2·3	4·7	7·0	30·2	14·0	41·9	—	43
May	—	0·8	2·5	27·5	34·2	27·9	7·1	120
June	—	—	—	0·8	27·7	68·1	3·4	177
July	—	—	—	—	10·3	88·5	1·3	39
Aug.	—	—	—	—	21·2	51·5	27·3	33
Sept.	—	—	12·3	36·3	0·9	12·3	38·2	106

September 1951–October 1952

	Gonad stages							
Month	I	II	III	IV	V	VI	VII	No. of gonads
Sept.	—	—	16·7	22·2	—	—	61·1	18
Oct.	—	14·3	21·4	14·3	—	—	50·0	14
Nov.	2·3	4·5	25·0	13·6	2·3	—	52·3	44
Dec.	—	—	—	37·5	—	—	62·5	8
Jan.	2·4	11·9	19·0	33·3	13·1	1·2	19·0	42
Feb.	6·0	1·2	20·2	46·4	16·7	7·1	2·4	84
Mar.	—	—	11·1	50·0	22·2	16·7	—	18
Apr.	1·1	1·4	2·8	37·9	43·3	13·2	0·3	178
May	—	—	1·3	10·2	51·6	36·8	—	152
June	—	—	—	1·4	56·4	40·0	2·1	70
July	—	—	—	6·7	15·6	62·2	15·6	90
Aug.	—	—	—	6·8	12·8	65·5	14·9	74
Sept.	—	—	—	4·1	8·2	58·2	29·6	49
Oct.	—	—	34·4	37·5	—	9·4	18·8	32

(B) SPAWNING MONTHS

(Samples arranged to show dates between which spawning occurred.)

	Gonad stages							
1951	I	II	III	IV	V	VI	VII	No. of gonads
15, 18 Aug.	—	—	—	—	25·0	75·0	—	4
31 Aug.	—	—	—	—	20·7	48·3	31·0	29
5–12 Sept.	—	—	—	12·0	—	38·0	50·0	25
21, 28 Sept.	—	—	16·0	43·8	1·2	4·3	34·6	81

	Gonad stages							
1952	I	II	III	IV	V	VI	VII	No. of gonads
9 July	—	—	—	—	26·3	73·7	—	38
17 July	—	—	—	9·1	—	45·5	45·5	11
21, 29 July	—	—	—	12·2	9·8	56·1	22·0	41
5 Sept.	—	—	—	5·7	11·4	72·9	10·0	35
11 Sept.	—	—	—	—	—	21·4	78·6	14

TABLE 4. PERCENTAGE OF ADULT SCALLOPS AT EACH STAGE OF MATURITY

(A) MONTHLY PERCENTAGE

(All samples in each month combined)

October 1950–September 1951

Month	Gonad stages III	IV	V	VI	VII	No. of gonads
Oct.	31·3	34·3	—	—	34·3	67
Nov.	26·3	62·5	6·9	—	4·4	160
Dec.	8·5	74·5	17·0	—	—	47
Jan.	1·2	36·8	54·0	8·0	—	201
Feb.	2·0	14·3	59·2	24·5	—	98
Mar.	0·3	4·4	32·9	62·2	0·1	343
Apr.	0·4	2·0	6·5	90·4	0·6	245
May	—	10·1	4·1	39·0	46·9	318
June	—	2·9	76·9	10·8	9·4	346
July	—	—	10·0	85·7	4·3	70
Aug.	—	0·7	23·4	25·2	50·7	141
Sept.	5·9	25·8	5·9	14·1	48·3	145

August 1951–October 1952

Month	Gonad stages III	IV	V	VI	VII	No. of gonads
Aug.	—	—	12·4	27·0	60·6	113
Sept.	9·4	30·2	4·0	13·9	42·5	260
Oct.	23·0	63·1	3·4	4·9	5·5	293
Nov.	9·2	53·9	31·4	4·5	1·0	191
Dec.	1·8	33·5	40·4	22·0	2·3	109
Jan.	1·0	15·1	55·4	28·3	0·2	288
Feb.	—	3·7	49·8	45·7	0·8	246
Mar.	—	1·5	17·6	79·9	1·0	259
Apr.	—	21·0	7·1	59·3	12·5	554
May	—	5·1	79·6	15·2	0·2	445
June	—	0·2	52·6	46·5	0·7	227
July	—	5·2	10·0	76·2	8·6	296
Aug.	—	7·1	10·7	71·1	11·1	140
Sept.	—	—	6·8	52·1	41·1	96
Oct.	43·5	42·7	—	2·4	11·3	62

(B) SPAWNING MONTHS

(Samples arranged to show dates between which spawning occurred)

1951	Gonad stages III	IV	V	VI	VII	No. of gonads
4 May	—	—	3·3	93·3	3·3	60
11 May	—	5·2	3·4	29·3	62·1	116
18 May	—	18·3	4·9	23·9	52·8	142
17, 24 July	—	—	10·0	85·7	4·3	70
15, 18 Aug.	—	1·8	45·5	34·5	18·2	55
23, 31 Aug.	—	—	9·3	19·2	71·5	86

1952	Gonad stages III	IV	V	VI	VII	No. of gonads
1–12 Apr.	—	0·3	6·6	92·5	0·5	286
15, 17 Apr.	—	—	3·6	41·6	54·8	83
25 Apr.	—	62·4	9·4	15·9	12·2	185
9 July	—	—	13·2	86·8	—	148
17–29 July	—	10·5	6·8	65·5	17·2	148
5 Sept.	—	—	11·0	83·1	5·9	59
11 Sept.	—	—	—	2·7	97·3	37

retained by juveniles and adults after spawning, the first recognizable stage in recovery was stage III (recovering). Recovery continued throughout the winter months, stages III and IV predominating in October and November, and stages IV and V in December. Half-full (stage V) gonads were abundant in the late winter, and by March or April most adult scallops had full (stage VI) gonads. The latter, however, still had considerable numbers of spermatocytes and growing oocytes in the follicles, so that, although a mass-spawning occurred in the spring, it was only partial. In 1951 this spawning occurred between 4 and 11 May, and in 1952 between 12 and 15 April (Table 4B). The spring spawning was followed at once by recovery, and, since only a proportion of the gonad contents had been shed, the resulting partially spent gonad resembled somewhat the stage III gonad, so that the first recognizable stage of recovery was stage IV. Recovery continued throughout the summer, through stages IV and V, so that by July most adult scallops again had full (stage VI) gonads, this time with few spermatocytes and growing oocytes. These then took part in another mass-spawning, which resulted, in most individuals, in the production of almost completely spent gonads. This spawning occurred at approximately the same time as that of the juveniles, i.e. late in August (actually between the 18th and 23rd in the adults) in 1951 and between 5 and 11 September in 1952 (Table 4B). In addition, between 9 and 17 July 1952, again at the same time as the juveniles, a few adult scallops spawned slightly, and recovered quickly to take part in the main September spawning. In 1951, a sample taken on 15 August showed that a similar slight spawning had occurred in a few scallops between 24 July and 15 August, again before the main spawning.

The majority of adult scallops, then, spawn together twice during each annual breeding cycle, partially in April or May ('spring' spawning), and more completely in late August or September ('autumn' spawning). There is also a minor 'summer' spawning in July or early August. Virgin and juvenile scallops differ from adults in having only one major spawning, in autumn, though juveniles show some evidence of a minor summer spawning. The autumn spawning is therefore by far the most important in terms of numbers of gametes released.

SPAWNING

The spawning of *Pecten maximus* was observed on several occasions in the laboratory. The genital products are passed out through the two main ducts, through the kidneys, and into the mantle cavity, whence they are emitted in a cloud through the exhalant opening of the shell. No violent flapping of the shell valves occurs, but they may open and close gently at intervals of 1 or 2 min. The eggs settle and form an orange layer on the bottom of the container, while the sperm become dispersed in the water and make it cloudy.

Both kidneys become filled with either eggs or sperm. Dakin (1909) stated that the smaller left genital duct, which leads into the left kidney, serves only the left-hand side of the testis in the neighbourhood of its opening. In my observations, however, both eggs and sperm were found to pass out through either kidney. Eggs and sperm are not shed at the same time, but usually within a few hours of one another, sometimes the eggs and sometimes the sperm being shed first. This was found to be true in *Pecten maximus* by Dakin (1909) and in *Chlamys opercularis* by Fullarton (1890). In about 4% of cases one or other of the two parts of the gonad remained unspawned.

On the only occasion on which the act of spawning was observed from the beginning, the ovary spawned in 45 min., and was followed at once by the testis, which became spent in 2 h. On several occasions both parts of the gonad spawned overnight.

The running gonad has a patchy appearance, having dull areas which have shed all their ripe products, and bright areas which still contain them.

Sections of a running testis (Plate II K) show follicles in varying stages of spending; most follicles are partly empty, having spaces in their centres, but, while these spaces contain many free spermatozoa, some spermatozoa are still arranged radially, as in the full gonad, with their tails pointing inwards. The ducts are wide, and their lumina contain spermatozoa. The duct walls contain a secretion which stains purple in haematoxylin, which is thought to facilitate passage of the genital products.

Sections of a running ovary (Pl. II L) show that some follicles are in a spent condition, and contain the remains of oocytes, while others are still full, and in some of these are seen ova which have undergone or are undergoing maturation. In these ova, the germinal vesicle has broken down, the cytoplasm is evenly distributed, and a spindle with chromosomes can sometimes be seen. The ovum is still polygonal, and possesses a conspicuous membrane. Other follicles have shed a few ova, and have a few loose in their lumina. The ducts are wider and contain mature ova, and a purple-staining secretion is sometimes present in their walls.

It was found that ripe oocytes, cut from the full gonad and placed in sea water, quickly become spherical, lose the germinal vesicle, and can then be readily fertilized artificially. Eggs passed naturally from the gonad into the surrounding water behave similarly. These facts suggest that contact with sea water might cause the onset of maturation. It is possible that the eggs are often passed out of the ovary before maturation, since, in an ovary which has commenced to spawn, many fully-grown oocytes still retain their germinal vesicles, and also that contact with sea water in the mantle cavity results in the dissolution of the nuclear membrane. The genital ducts in the running gonad are so wide that water from the mantle cavity and kidneys might conceivably pass along them to the follicles, and cause the onset of maturation in some oocytes before they leave the follicle. Coe (1933) found such a state of affairs in *Teredo*, with sea water causing the initiation of maturation.

Tang (1941) stated that *Pecten* off Port Erin began to spawn in numbers when the water temperature reached 10° C, but this is contradicted by the present study. Of the six spawnings which occurred in 1951 and 1952 it was possible in five cases to state, to within a few days, when spawning had occurred (the exception was the small summer spawning of 1951, when insufficient samples could be taken). Spawning was not caused by the attainment of a definite temperature, but occurred at several temperatures between 7·2 and 13·7° C, viz. spring 1951—7·2° C, autumn 1951—13·7° C, spring 1952—8·1° C, summer 1952—12·7° C, autumn 1952—13·5° C.

FERTILIZATION

Fertilization is external, and, at the times of the mass spawnings, there are relatively high concentrations of genital products in the sea, greatly increasing the chances of successful fertilization.

In the laboratory, artificial fertilization of eggs cut from the gonad was readily carried out. In nature, where each individual sheds its two types of genital products separately into the sea, cross-fertilization must be the general rule. Experimentally, however, cross- and self-fertilization were obtained with equal facility. On one occasion a rough count showed that about 80% fertilization was obtained, though usually the percentage was much lower than this. Better results were obtained using ripe gametes which had been shed normally by the scallop.

Normally only mature eggs from a full gonad are capable of being fertilized. However, sperm from stage IV, V and VI gonads, as well as residual sperm from partially spent gonads, are capable of fertilizing them.

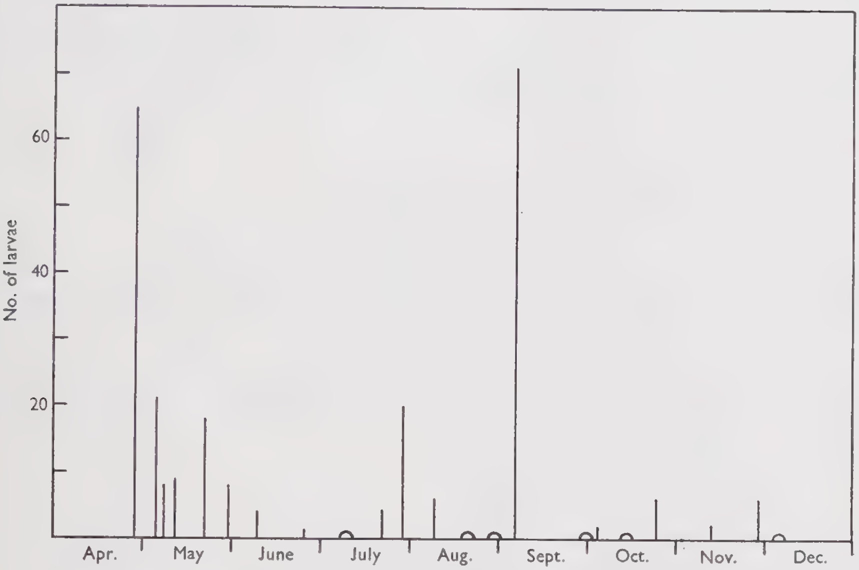
LARVAE AND SPAT

No description of the larva of *P. maximus* as such was found in the literature. Rees (1950) figured and described various pectinid larvae, but was unable to allocate the larvae to particular species.

Plankton samples were taken off Bay Fine, just below the surface, using a fine townet, 129 meshes to the inch, at intervals of 1–2 weeks during the period 28 April–24 December 1952. Dr Rees very kindly examined any pectinid larvae present, together with the prodissoconchs of recently settled spat, and as a result he was able to identify his pectinid E as the larva of *P. maximus* (Rees, 1954).

Text-fig. 5 shows the numbers of larvae in the samples. There are three main peaks, two large ones on 28 April and 5 September, and a smaller one on 29 July. The first large peak would probably be composed of larvae arising from the spring spawning, the small peak of larvae from the small summer partial spawning, and the second large peak of larvae from the autumn spawning. Since the mass spawning of scallops in the population studied did not occur until between 5 and 11 September, the larvae found on 5 September probably originated from the spawn of a different group of scallops in the vicinity, which had spawned somewhat earlier.

The large numbers of larvae in the plankton haul of 28 April show that the spring spawning resulted in the successful production of larvae. When spawning was recorded, between 12 and 15 April, the temperature was 8·1° C, indicating that a temperature of 10° C is not minimal for the production of larvae, as was thought by Tang (1941).



Text-fig. 5. Numbers of larvae in plankton samples, April-December 1952. Blank hauls are indicated by semicircles.

The sampling was not adequate to show the exact times and compositions of the peaks in Text-fig. 5, this being especially so in the September peak, when there was a gap of 25 days after the sample of 5 September. The September peak would be expected to be much higher than the April peak, owing to the much greater amount of spawn shed in the autumn than in the spring spawning (see above, p. 664).

Elmhirst (1945) stated that the larvae of *P. maximus* settle on *Laminaria saccharina*, while Hertling (1934) found a few young specimens attached to a drifting trunk off Heligoland. On only one occasion, however, did I find the spat of *Pecten maximus*, namely on 29 August 1952, when two specimens, 3·0 and 3·5 mm long respectively, were found on *Desmarestia* on the Bradda bed. These presumably arose from the small summer spawning. In addition, Mr T. B. Bagenal kindly sent me a few young specimens which he found attached to *Laminaria saccharina* at Millport. My inability to find more young *Pecten maximus* was surprising in view of the large numbers of newly settled *Chlamys opercularis* which I found attached to *Laminaria saccharina*.

The two recently settled individuals of 3·0 and 3·5 mm found off Port Erin were easily recognized, since the shape of the young shell can be deduced from the concentric striae on the adult shell (Mason, 1957). The shell was transparent and almost colourless, with a conspicuous byssal notch, and both shell valves were convex. Larger specimens, some 5–25 mm long, possess a left valve which is concave except for a small convex area near the umbo, while the right valve is convex. Ribs begin to appear 8–10 mm from the umbo, and the shell then begins to take on the adult form, with a flat left valve. The byssal notch has almost disappeared after about 2 years, when the scallop is about 50–55 mm long.

DISCUSSION

From an economic standpoint it is useful to know that *Pecten maximus* first becomes sexually mature and spawns in the summer after the deposition of the second annual growth-ring on the shell. In the type of commercial dredge used round the Isle of Man, almost all the scallops caught are over 100 mm long, a length which they attain on the average in the autumn after the deposition of the third ring (Mason, 1957). Thus almost all the scallops caught commercially are adults, and have spawned at least twice, while some will have spawned more than twice.

The only parts of the scallop which are eaten are the adductor muscle and the gonad, so that the scallop is most valuable commercially when the gonad is full. A scallop of reasonable commercial size, say 120 mm long, with a full gonad, yields on the average approximately 32 g of edible material, of which the muscle accounts for 24 g and the gonad for 8 g. Thus scallops are in the prime of condition in the months immediately prior to spawning, namely March–April and July–August, but they are not normally caught in the summer months owing to the difficulty of keeping them in good condition in hot weather during transport to London, the main market.

The occurrence of two main spawnings yearly among adult scallops has not been noted by previous workers in Manx waters, though recently, since the present work was completed, Gibson (1956) has noted it in *P. maximus* in Irish waters.

With the occurrence of two main spawnings can be correlated the fact that two types of first year's growth can be recognized on the shell, according to the length of time available for growth before the first winter cessation (Mason, 1957). Autumn-spawned scallops are greatly in the majority owing to the much greater amount of spawn released then as compared with the spring spawning.

In the system of classification used by Coe (1945), *P. maximus* is a 'functional hermaphrodite', in which both types of sexual cells are produced at the same time, but are not usually discharged together. Coe (1945) stated that in the genus *Pecten* there is a strong tendency towards protandry, that in the young gonad spermatogenesis may be in progress long before the ova have begun the deposition of yolk, and that some or all of the sperm is discharged before the eggs are fully ripe. Pelseneer (1935) said that hermaphrodite molluscs in general are protandrous, the sperm being ripe before the eggs, so that self-fertilizing hermaphrodites are rare and cross-fertilization is the general rule. Dalmon (1938) went so far as to say that *P. maximus* is so distinctly protandrous as to render self-fertilization impossible. The results of the present study contradict Dalmon's statement and support one made by Coe (1945) that, experimentally, self-fertilization in fully-ripe functional hermaphrodites seems to take place as readily as cross-fertilization.

The present study has shown that, in the virgin gonad of *P. maximus*, there is an early tendency, not towards protandry, but towards protogyny, in that oocytes are first produced before spermatocytes (Table 1). Later, however, this tendency is reversed, and spermatogenesis so far outstrips oogenesis that the male follicles contain some spermatozoa while the female follicles still contain only half-grown oocytes. Similarly, after spawning, spermatogenesis occurs more rapidly than oogenesis, and many sperm are present long before the oocytes are fully grown. These sperm from the earlier gonad stages are physiologically ripe and capable of fertilizing ripe eggs which, however, occur only in the full (stage VI) gonad. The sperm are stored in the testis until the gonad is full, and the products of the two sexes are discharged separately, but within a few hours of each other, often some weeks after the full stage is reached. The products of either sex may be shed first, so that some individuals are functionally protandrous and others are functionally protogynous.

A retention of morphologically ripe gametes in the gonad for considerable periods of the year was also found in *Venus mercenaria* by Loosanoff (1937). *Pecten maximus* also resembles *Venus mercenaria* in that there is no resting period in the gonad after spawning, and that gametogenesis occurs over most of the year, even in the autumn and winter months when the temperature is falling. Loosanoff considered these phenomena as unusual among lamellibranchs.

This paper is based on parts of a thesis[1] presented to the University of Liverpool in 1953 for the degree of Ph.D. The work was carried out at the Marine Biological Station, Port Erin, while I held a Herdman Studentship

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[1] Investigations on the scallop [*Pecten maximus* (L.)] in Manx waters, 1953.

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SUMMARY

Pecten maximus is a functional hermaphrodite, whose gonad consists of separate male and female parts. Its breeding cycle in Manx waters was determined by examination of the macroscopic and microscopic changes in the gonad. The virgin scallops spawn in the autumn after the deposition of the second growth ring on the shell. In the next year, as juveniles, they have one main mass spawning, in autumn, and a very small, partial one earlier in the summer. In all subsequent years, as adults, they have two mass spawnings, a partial one in spring and a more complete one in autumn, while a much smaller, partial one occurs in the summer. Gametogenesis occurs throughout the year, and there is no resting period after spawning.

P. maximus shows an early tendency to protogyny in the development of the virgin gonad, but this is soon reversed, and a tendency to protandry appears, which is also apparent in the recovery after spawning, so that ripe sperm are present long before fully-grown oocytes. These ripe sperm are retained until the gonad is full, and the two parts of the gonad spawn separately within a short time of each other, the whole act of spawning taking a few hours. The products of either sex may be shed first. While cross-fertilization must be the rule in nature, experimentally both cross- and self-fertilization of mature eggs, which had lost their germinal vesicles, were obtained easily.

Spawning does not take place at a definite temperature.

The numbers of larvae in plankton hauls show three peaks, two large and one small, corresponding to the two main and one small spawnings. No reason can be advanced for my inability to find more than a few recently settled individuals.

Almost all scallops caught commercially have spawned at least twice. Scallops are most valuable commercially when their gonads are full prior to spawning.

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A METHOD FOR THE MEASUREMENT OF VITAMIN B_{12} CONCENTRATION IN SEA WATER

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(Text-fig. 1)

The vitamin B_{12} concentration in sea water is so very low that only the most sensitive microbiological assay techniques are likely to be capable of measuring it, but the salt concentration is so high as to be inhibitory to most of the organisms conventionally used for the microbiological assay of the vitamin. Nevertheless, the consideration that vitamin B_{12} concentration may have an influence on the amounts and types of life present in sea areas has persuaded several investigators to attempt its measurement.

The methods so far employed have each their own advantages and disadvantages (Droop, 1954, 1955; Lewin, 1954; Sweeney, 1954; Cowey, 1956; Adair & Vishniac, 1958). The method to be described here offers advantages in sensitivity, permitting direct measurement of the vitamin in small quantities of oceanic waters after further dilution; samples to be measured pass through comparatively few different containers and thus there is less risk of their accumulating adventitious vitamin; simple and easily cleaned apparatus is used, and much less bench-work is involved than when methods other than dilution are used for lowering the salt concentration.

I am deeply indebted to Dr L. R. Fisher and Dr S. K. Kon who introduced me to the subject of vitamins in the sea and their possible significance, and to Dr J. E. Ford for his advice on microbiological techniques.

Thanks are also due to Dr G. I. M. Ross, then at the Vincent Square Laboratories, Westminster Hospital, who provided the culture of *Euglena gracilis* strain *z* with details of the growth medium before their publication; and to Dr R. Johnston of the Scottish Home Department Marine Laboratory, Aberdeen, for providing sea-water samples.

MATERIAL AND METHODS

Principle

The organism used is the freshwater flagellate *Euglena gracilis* strain *z*, grown in the medium of Hutner, Bach & Ross (1956). Carsted (cited by

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* Grant-aided by the Development Fund.

Hutner, Bach & Ross, 1956) found that this organism shows a growth response not only to cyanocobalamin (the form of vitamin B_{12} active for man and other higher animals), but also to two of its analogues, pseudovitamin B_{12} and Factor A. In this it resembles *Monochrysis lutheri* (Droop, 1955) but differs from some other marine algae (Droop, 1957). In his 1957 paper, Droop mentions that in the sea the analogues of vitamin B_{12} may have an importance equal to that of the vitamin.

The assay is carried out in a manner rather different from that described by Hutner *et al.* (1956); instead of measuring the growth of the *Euglena* population turbidimetrically, the chlorophyll from the cells is extracted and measured colorimetrically by the procedure of Heinrich & Lahann (1952). Various chlorophyll-measuring techniques resembling this procedure have of course long been familiar to marine biologists, who use them to obtain estimates of the phytoplankton standing crop in the sea (e.g. Harvey, 1934; Atkins & Parke, 1951).

Chlorophyll production was found to be proportional to the amount of vitamin B_{12} supplied to the *Euglena* population during its growth; which provides an exceptionally sensitive index of the vitamin concentration.

The sea-water sample has to be diluted with distilled water before use in order to reduce its salt concentration to a level which will not interfere with the growth of *Euglena*.

Several different dilutions of the sea-water sample, and at least two flasks of each dilution, are assayed together. Other flasks containing known amounts of vitamin B_{12} are necessary in each assay to provide the calibration.

Cleaning of glassware

Careful cleaning of the glassware for use in the assay is essential, as otherwise it might contribute significant amounts of vitamin B_{12}; perhaps even more than would be contained in the sample itself. The extent to which the glassware can be freed of vitamin B_{12} may well be the factor which imposes a practical limit on the accuracy and sensitivity of the assay.

Conical flasks of 50 and 100 ml. capacities are used. Before being brought into use for sea-water assays, flasks are filled with an approximately 10% (w/v) solution of sodium hydroxide and then steamed. They are then washed in turn with distilled water, dilute hydrochloric acid and several changes of distilled water.

With flasks used for sea-water assays and no other purpose, it has been found sufficient to wash them in a dilute detergent (Lissapol; I.C.I.) followed by distilled water acidulated with hydrochloric acid; then to fill them with distilled water and autoclave them, repeating this two or three times. The clean flasks are left in a drying oven until they are needed.

Preparation of medium

The medium recommended and described by Hutner *et al.* (1956) is prepared in solution at five times single strength, and stored in Polythene bottles at −20° C.

Preparation of sea water

In the procedure to be described the assay is set up with four graded dilutions of the sample, each in triplicate. For such an assay 10 ml. of sea water are sufficient. Amounts for assays to be done with greater replication to provide higher precision can readily be calculated.

While awaiting assay, the sea water is stored cold with approximately 1 % of the preservative of Hutner & Bjerknes (1948) (1 part by volume *o*-fluorotoluene, 1 part dichloroethane, 2 parts *n*-butyl chloride).

10 ml. of the sea water are placed in a 100 ml. conical flask, together with 0·5 ml. citrate-phosphate buffer of pH 4·6 (McIlvaine, 1921*) and 0·5 ml. of a freshly prepared 0·001 % solution of NaCN. 60–80 ml. of glass-distilled water are then added, and the flasks heated in the autoclave in flowing steam for 15 min. This treatment eliminates any volatile preservative present; and any vitamin B_{12} not originally in the free cyano form is converted into it (Ford, 1952; Coates & Ford, 1955). In the free cyano form, the vitamin is relatively stable, and nutritionally available to the assay organism.

After the steaming, the flask is cooled and the contents made up to 100 ml. with distilled water, which represents a 1 in 10 dilution of the original sea water.

This degree of dilution has been found to be usually suitable for bringing samples within the sensitivity range of the assay. A lesser degree of dilution should if possible be avoided as higher salt concentrations interfere increasingly with the growth of *Euglena* (Hutner *et al.*, 1956).

Layout of assay.

This prepared sea water is now distributed into twelve flasks as follows:

2 ml., 4 ml., 8 ml., and 16 ml. portions respectively into each of three flasks

To each of these flasks are then added 4 ml. of the five-times strength medium and, where necessary, distilled water to make up the volume to 20 ml.

Parallel series of flasks are prepared containing graded amounts of cyanocobalamin within the concentration range of 0·025–0·20 $\mu\mu$g/ml. For this standard range a solution containing 0·25 $\mu\mu$g cyanocobalamin/ml. distilled water is prepared by serial dilutions with distilled water from a stock solution containing 5 μg cyanocobalamin/ml. 25% aqueous ethyl alcohol, and is distributed as follows:

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* McIlvaine's buffer is described in laboratory handbooks, e.g. Vogel, 1939.

2 ml., 4 ml., 8 ml. and 16 ml. portions respectively into each of three flasks

Medium and distilled water are then added as to the test extracts.

At least one other flask is made up to contain medium and water only, with no added vitamin B_{12}. This flask provides the experimental blank in the subsequent colorimetric measurement.

The flasks are closed with tight-fitting metal caps,* or are plugged with non-absorbent cotton-wool and covered with greaseproof paper caps. They are then autoclaved at 10 lb. pressure for 10 min.

Preparation of inoculum

For the *Euglena* inoculum 4–7-day-old cultures are used, grown at room temperature under approximately 200 f.c. illumination in 10 ml. portions of single-strength medium containing 80 μμg cyanocobalamin/ml. Such cultures contain about 10^4 cells/cu.mm.

Before use, the inoculum culture is washed so as to reduce carry-over of vitamin B_{12}, and to remove the inhibitory factor present in the culture liquors (Kristensen, 1955; cf. Ford, Gregory & Holdsworth, 1955).

This washing is performed by centrifuging down the cells and decanting the growth medium. The cells are re-suspended in sterile single-strength medium containing no vitamin B_{12}, and are again centrifuged and the medium is discarded. This procedure is repeated and the cells are finally suspended in 10 ml. of the single-strength medium containing no vitamin B_{12}.

The washing procedure is carried out under aseptic conditions to minimize risk of contamination of the *Euglena* culture.

Inoculation and Incubation.

The assay flasks, cooled to room temperature after the autoclaving, are each inoculated with one drop of the washed *Euglena* suspension. During this inoculation care must again be taken to prevent contamination by air-borne organisms.

The flasks are then incubated for about 10–12 days under 180 f.c. illumination and at approximately 28° C.

During the period of incubation the flasks are daily given a brief shaking to break any clumping of the *Euglena* cells. They are put back in random rearrangement.

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* Moncrieff Ltd. of Perth supply flasks suitable for use with ‘Oxoid’ caps.

Reading of assay.

After incubation, the contents of each flask are poured into a centrifuge tube, the *Euglena* cells centrifuged down, and the medium decanted; 3 ml. acetone are added to each tube and the cells stirred up. Chlorophyll leaves the cells and goes into solution. The cell debris is again centrifuged down and is extracted with a further 1 ml. acetone; this extract is added to the first. The volume is finally made up to 4 ml. just before the colorimetric measurement to compensate for any evaporation of the acetone.

The chlorophyll extract from the *Euglena* grown in one of the flasks containing no added vitamin B_{12} is used as the blank in the measurement. The intensity of the colour of each chlorophyll extract is read against this blank. In this laboratory a Beckman DU spectrophotometer with 1 cm Corex cells is used, the optical density reading, log I_0/I at 432 mμ, being taken.

Calculation of results

The optical density readings deriving from the standard series of cultures grown with known amounts of cyanocobalamin are used to construct a calibration curve (Fig. 1). The concentration of cyanocobalamin corresponding to the optical density readings obtained from each of the dilutions of the sea-water sample is then read from the curve, and an average value for the cyanocobalamin concentration in the sea water is calculated.

Internal standards

The extent of agreement between results calculated from the optical density measurements at different sample dilutions gives an indication of their validity. It can be further checked by the use of an 'internal standard'. If an internal standard is to be employed, the procedure for setting up the assay is modified as follows: before the final dilution step in the preparation of the sea-water sample, two equal portions are taken and to one is added a known amount of cyanocobalamin. Both portions are then made up to the same volume with distilled water to provide the solutions for two series of assay flasks. A comparison of these two series makes possible the calculation of the vitamin B_{12} concentration originally present in the sea water without reference to the 'external' (cyanocobalamin in distilled water) standard.

Agreement between the values calculated by means of such an internal standard and those obtained with reference to the external standard provides good evidence for the validity of the result.

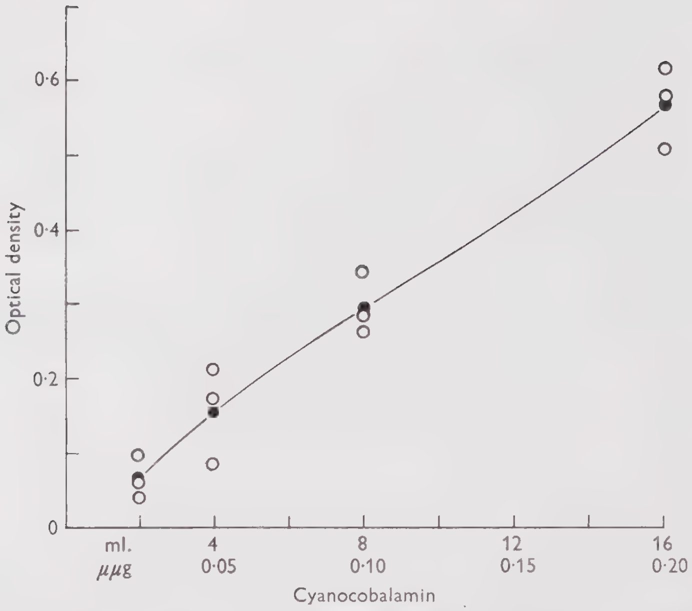

Fig. 1. Standard curve for assay of vitamin B_{12} by *Euglena gracilis* strain *z*. Standard cyanocobalamin 0·25 μμg/ml.; incubation for 12 days in this instance. ○ ○, values for individual flasks; ● ●, mean values. A smooth curve is drawn through the mean optical density values at the different levels of vitamin concentration. Slope and shape of the curve vary slightly from assay to assay. The horizontal ml. units refer to standard solution per flask, and the μμg units to cyanocobalamin per ml. The vertical units represent optical density of chlorophyll extract at 432 mμ.

TESTS OF THE *EUGLENA* ASSAY METHOD

Comparison with a previous method

Two 4 l. samples of surface sea water, collected 7 March 1956 at 58° 40′ N., 6° 10′ W. were supplied for assay by Dr R. Johnston of the Scottish Home Department Marine Laboratory, Aberdeen.

TABLE 1. COMPARISON OF METHODS OF MEASURING VITAMIN B_{12} IN SEA WATER

(Two surface samples taken 7 March 1956 at 58° 40′ N., 6° 10′ W.)

	Assay method			
	Dilution; *E. gracilis z* Vitamin B_{12} in mμg/l.		Phenol passage; *Lb. leichmannii* Vitamin B_{12} in mμg/l.	
Sample	By external standard	By internal standard	By external standard	By internal standard
A	2·0	1·6	1·7	1·5
B	1·5	1·4	1·0	1·0

10 ml. portions of each were assayed as described; 3500 ml. portions of each were subjected to the techique used by Cowey (1956) of phenol passage and subsequent assay with *Lactobacillus leichmannii*. The results (Table 1) show that agreement between the results from the different methods was reasonably close. The accompanying paper (Daisley & Fisher, 1958) lists six more examples of sea water assayed by both the *Euglena* method and the method of Cowey (1956), as well as results for thirty-five other samples assayed by the *Euglena* method alone.

Variation between repeated assays of samples during storage

Three samples of sea water, also supplied by Dr Johnston, were stored for 9 months and assayed four times at intervals during that period. Each assay was carried out with the same degree of replication as that described in this paper, and results were calculated with reference to external standards. The results (Table 2) give no indication of a fall in the vitamin B_{12} concentration during storage; they do, however, show some variation between results from different assay batches. The degree of variation is greater than is customarily obtained with other methods discussed by Coates & Ford (1955), but this may result from the higher sensitivity of the *Euglena* assay, which will make it more susceptible to the effects of adventitious vitamin B_{12} and to slight variations in the experimental procedure.

TABLE 2. VARIATION BETWEEN REPEATED ASSAYS OF SAMPLES OF SEA WATER DURING STORAGE

(The figures refer to vitamin B_{12} concentrations as measured with *Euglena gracilis* strain *z*)

Sample collected:	Time	7 Mar. 1957	13 Mar. 1957	15 Mar. 1957
	Place	57° 02′ N. 1° 45′ W.	58° 30′ N. 0° 30′ E.	59° 25′ N. 4° 00′ E.
	Depth	10 m	10 m	10 m
		Vitamin B_{12} in mμg/l.		
Assay batch	25 Apr. 1957	1·6	1·4	0·9
	21 May 1957	1·2	1·4	0·8
	7 Aug. 1957	1·9	1·8	1·2
	9 Dec. 1957	1·4	1·1	1·0

The precision of the assay method is, however, adequate for showing any considerable variation in vitamin B_{12} concentration between different samples, such as the several-fold differences between the concentrations in coastal and oceanic waters or between summer and winter water (Cowey, 1956), or water from different depths (Daisley & Fisher, 1958).

DISCUSSION

It is probable that assays with other chlorophyll- (or other pigment-) producing organisms, such as those mentioned by Droop (1954), could be increased in sensitivity by the procedure described: starting with a washed depleted inoculum, growing a large volume of the organism, and then extracting its pigment into a small volume of solvent and measuring the colour intensity obtained.

If there were available a range of organisms each responsive specifically to a different member of the vitamin B_{12} family of compounds, it would be possible to make separate estimates of the concentrations of each such compound present in the sea; but it is an interesting fact that those organisms so far investigated which need members of the vitamin B_{12} family all respond to cyanocobalamin itself, in addition to any other analogue which they may utilize (see the lists given by Coates & Ford, 1955; Droop, 1957).

Some degree of differentiation would, however, be possible by comparison of results obtained with a cyanocobalamin-specific organism and an organism responding to several of the analogues, as was done by Cowey (1956) following the work of Ford (1953).

Not all the vitamin B_{12} which, in assay procedures such as that described in this paper, is made available to the assay organism is necessarily in a free and nutritionally active form in the original sea water. Some or even all may be bound in cells or cell fragments, or be combined with other substances. To differentiate between 'free' and 'bound' vitamin B_{12} in sea water, more complex procedures would have to be applied, as was found necessary with milk samples (Gregory, 1954); and if some proportion of the vitamin B_{12} in the sea was found to be in 'bound' forms, there would remain the problem that these might be differently available to different marine organisms.

Further work on these various aspects discussed is continuing.

Results obtained with the *Euglena* assay method with samples taken to study the vertical distribution of vitamin B_{12} in the sea are presented in a companion article (Daisley & Fisher, 1958).

SUMMARY

A sensitive method for the measurement of vitamin B_{12} concentration in sea water is described.

Small volumes, e.g. 10 ml., of sea water are diluted with distilled water and a microbiological assay using the fresh-water flagellate *Euglena gracilis* strain *z* is carried out. The diluted sea-water samples are enriched with a growth medium, washed inocula of the *Euglena* are added, and these cultures are grown for up to 2 weeks under constant conditions of temperature and illumination. The chlorophyll is then extracted from the cultures into small volumes of solvent and the optical densities measured. These optical density readings are related to the concentrations of vitamin B_{12} available to the *Euglena* populations during their growth.

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VERTICAL DISTRIBUTION OF VITAMIN B_{12} IN THE SEA

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A considerable seasonal variation in the vitamin B_{12} concentration in oceanic surface water has been demonstrated by Cowey (1956).

The present investigation was undertaken to examine any variation with depth.

The samples were taken in the Bay of Biscay during cruises of the R.V. 'Sarsia' in May and June 1956 and May 1957. Stations sampled were:

Station A:	47° 30′ N.,	7° 18′ W.
Station C:	46° 30′ N.,	8° 00′ W.
Station 15:	48° 25′ N.,	9° 57′ W.
Station 16:	48° 24′ N.,	10° 00′ W.
Station 17:	48° 24′ N.,	9° 53′ W.
Station 19:	47° 47′ N.,	9° 18′ W.

The water was collected in a Nansen Pettersson bottle and in Nansen reversing bottles, then filtered through Whatman No. 3 filter-paper, preserved and assayed by the *Euglena* method as already described (Daisley, 1958). The 1956 samples were assayed twice each and the mean values are given in Table 1; the 1957 samples were assayed once only.

In addition, some of the 1956 samples, which were larger (but consequently fewer) than the 1957 samples, were also assayed by the Cowey (1956) procedure as a check on the accuracy of the *Euglena* method.

The results are given in Table 1. For the depth calculations and oxygen measurements we are indebted to Dr L. H. N. Cooper and Mr F. A. J. Armstrong of the Plymouth Marine Laboratory. They calculated the oxygen saturation values by reference to the tables of Truesdale, Downing & Lowden (1955).

The 1956 results indicated an inverse relationship between the vitamin B_{12} concentration and the oxygen concentration, suggesting that perhaps the vitamin was being synthesized in association with respiratory activity by organisms in these waters. The more detailed 1957 survey showed, however, that although the vitamin B_{12} concentration is in general highest at intermediate depths (*ca.* 200–2000 m) and the oxygen concentration reaches its minimum values within this region, the inverse relationship between them is not a close one.

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TABLE 1. VERTICAL DISTRIBUTION OF VITAMIN B_{12} IN THE SEA

				Vitamin B_{12}	
Date station and sounding	Depth (to nearest 10 m) (m)	Oxygen concentration (ml./l.)	Oxygen saturation (%)	Daisley's method (*E. gracilis z*) (mμg/l.)	Cowey's method (*Lb. leichmannii*) (mμg/l.)
28 May 1956 C 4710 m	100	5·74	97·4	0·8	0·7
	500	4·92	81·9	2·1	—
	880	4·20	68·8	2·2	—
	990	4·40	71·3	4·6	4·2
	2110	5·51	77·1	3·0	—
29 May 1956 C 4710 m	0	—	—	0·6	—
20 June 1956 A 1260 m	20	6·44	113·4	0·6	0·4
22 June 1956 C 4710 m	4040	4·82	65·0	0·5	0·5
23 June 1956 A 1260 m	600	—	—	2·5	1·7
	1190	4·52	71·1	1·1	1·5
5 May 1957 15 590 m	220	5·76	97·0	0·3	—
	310	5·61	94·3	1·9	—
	400	5·31	88·8	1·9	—
	490	5·15	85·8	1·9	—
	580	4·93	81·5	1·7	—
5 May 1957 16 2620 m	1270	4·93	74·9	1·0	—
	1360	5·03	75·2	2·6	—
	1450	5·20	76·9	5·0	—
	1540	5·31	78·1	5·0	—
	1630	5·09	—	2·0	—
5 May 1957 17 2100 m	400	5·41	90·6	1·5	—
	490	5·05	83·9	1·7	—
	590	4·82	78·6	3·8	—
	690	4·83	78·7	3·5	—
	790	4·81	78·0	2·8	—
	860	4·48	72·7	2·4	—
	950	4·55	72·9	2·3	—
	1050	4·61	73·4	1·6	—
	1140	4·72	74·2	1·0	—
	1240	4·90	75·3	1·6	—
6 May 1957 19 3900 m	0	—	—	0·3	—
	190	5·72	96·5	2·0	—
	370	5·22	87·1	4·0	—
	560	4·82	80·1	1·6	—
	770	4·47	—	0·2	—
	970	4·26	68·5	1·5	—
	1170	4·61	71·4	3·9	—
	1560	5·77	82·4	1·6	—
	1980	5·74	80·3	1·0	—
	2980	5·44	74·3	0·8	—
	3580	5·38	72·7	0·4	—
	3600	5·26	71·1		

Dr Cooper intends to publish later other hydrographic data relating to this sampling programme; these other data do not, however, display any correlations with the pattern of vitamin B_{12} distribution.

No samples, even those from the greatest depths, were entirely lacking in vitamin B_{12}; the possibility must be considered, however, that the small amounts of vitamin B_{12} recorded in the deepest samples may derive from adsorptive contamination of the bottles as they passed down through the vitamin-rich regions. A means of avoiding any such possible source of experimental error will be sought.

When this present work was completed, we found a recent report of a survey of vitamin B_{12} concentration in the sea near Japan; here also a considerable variation with depth had been found, but no samples were from below 1200 m (Kashiwada, Kakimoto, Morita, Kanazawa & Kawagoe, 1957).

No conclusion can as yet be drawn regarding the cause of the variation in vitamin B_{12} concentration; possible contributory factors may be water movements, bacterial activity, consumption of the vitamin by the inhabitants of the illuminated zone and sinking of such organisms and their disintegration at lower levels.

We are grateful to the Director and staff of the Marine Biological Association for facilities and advice and especially to Dr L. H. N. Cooper and Mr F. A. J. Armstrong for their help in the collection of samples and for the provision of hydrographic data.

SUMMARY

Sea-water samples were taken from various depths in the Bay of Biscay in May and June of 1956 and 1957.

Low vitamin B_{12} concentrations were found in the upper illuminated zone and in the greatest depths (mean value for seven samples, 0·57 mμg/l.; standard deviation 0·19 mμg/l.), whereas at intermediate depths (190–2110 metres) the values were generally higher, up to 5·0 mμg/l. in two instances (mean value for 34 samples, 2·26 mμg/l.; standard deviation 1·22 mμg/l.).

Evidently there exists a considerable variation with depth of the vitamin B_{12} concentration in the sea region sampled at this season. It remains for further research to show what causes this vertical variation to exist, and what influence, if any, it has on life in these waters.

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SUBLITTORAL ALGAL POPULATION IN PORT ERIN BAY, ISLE OF MAN

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(Text-fig. 1)

In recent years much information has accumulated on the composition and distribution of algal populations in the Isle of Man, with increased knowledge of their ecological relationships (Knight & Parke, 1931, 1950; Jones, 1948; Lodge, 1948, 1954; Burrows & Lodge, 1949, 1951; Southward, 1953). This, however, has mostly come from studies made in the intertidal region, and even here many problems remain for which, at present, no satisfactory explanation can be found. The mostdifficult of these, perhaps, relates to the appearance of algal species for a limited period of the year only: it is not known where and in what form their life-histories are continued for the rest of the year. The littoral and sublittoral regions are known to have many species in common. This means that the differences between the regions, as environments for the growth of algae, can be bridged by the plasticity of the species concerned. It seemed likely, therefore, that a detailed study of the sublittoral algal populations might help towards an understanding of the ecology of both regions and also of the interactions between them.

Attempts have been made by several workers using diving equipment to find the exact distribution of sublittoral algae (Kitching, Macan & Gilson, 1934; Bursa & Wojtusiak, 1939, 1948; Waern, 1952; Forster, 1955, 1958; Aleem, 1956), but the scope of such work is limited at present. Walker (1947) made surveys of sublittoral algae around the coast of Scotland using a specially designed view-box for inspection of the sea floor and a calibrated spring grab for collecting samples from fixed points, and was able to make quantitative estimates of the distribution of the larger brown algae over a wide area.

In August 1955 a survey of the sublittoral algae of Port Erin Bay was begun using a combination of the techniques mentioned above. The bay is approximately half a mile square and partially bounded on the seaward end by a broken breakwater. On the north and south the sides are rocky, while most of the floor of the bay is sandy. From the beach at the eastern end to the breakwater and open sea on the west, the bay shelves gradually to a depth of 12 m. The deepest part lies in the angle formed by the breakwater and the south shore and reaches a depth of 14 m. The limit of the sublittoral rock surface was determined in 1956 by echo-sounding. The limit was taken as the point at which the steeply sloping side of the bay turned abruptly to the horizontal. As far as possible this was checked by direct observation but, at the time this work was carried out, the water was not very clear. Tidal currents enter the bay on the north side and swing round towards the south, but little is known about water movement on the floor of the bay. The area was sampled by means of a spring grab of the type used by Walker (1947). It took algae from a square of side 75 cm and was used much in the same way that an ecologist on land uses a quadrat as a sampling unit. The grab was worked from the motor-boat 'Cypris' and, for each sample taken, the boat position was determined by taking sextant readings on to accurately charted points on the shore. Depths were measured with a lead line, and, so that it would be possible to relate all depths to a fixed tide level, simultaneous readings were made on a tide-staff on the harbour wall for all periods over which samples were taken in the bay. Through an analysis of the samples it was hoped to work out the over-all distribution of the algae. The action of the grab was watched through the very calm clear water, and observations on its action on different types of substrate were also made by a diver using an aqualung. On a number of occasions the diver completed clearance of squares incompletely cleared by the grab. In this way it was found that the grab did not always give a complete clearance of the sample squares and undoubtedly a good deal was missed, but the method had the advantage that, for the algae collected, the exact positions in which they were growing were determined.

Because of the extremely favourable weather conditions in 1955, the survey proved to be much easier than had been anticipated. The water was so calm and clear that the major beds could easily be seen through a glass-bottomed bucket and their limits were plotted from a rowing boat with the help of a sextant. The survey was repeated in August 1956 using the same general methods, and 125 samples were taken on each occasion.

DISTRIBUTION OF THE SUBLITTORAL ALGAE

It was found that there were two distinct algal vegetation regions in the sublittoral, associated with the two main types of substrate, continuous rock surface and sand. The bay floor consists mainly of sand overlaid by stones and boulders of varying sizes; in places these lie close together in large groups, in other places they are widely scattered and often partially buried in the sand. No doubt much movement of stones and boulders occurs on the sea bed at some seasons of the year, but many boulders brought up by dredge and grab appeared to have been buried for some time and supported growths of algae only on one surface. Certain large groups of boulders are conspicuous at all times of the year as a result of the weed they carry. An analysis of the composition of the vegetation associated with the two regions is given in Table 1. Only those species, the identification of which is certain, have been included. Herbarium specimens of the algae collected during the survey have been deposited in the herbarium of the Botany Department of Liverpool University. The names used are those in Parke's check-list (1953), with later corrections (Parke, 1956, 1957).

The vegetation of the continuous rock surface

The algae attached to the rock surface form a more or less permanent population conspicuous at all times of the year. In this population *Laminaria digitata*, *L. saccharina*, *L. hyperborea* and *Saccorhiza polyschides* are the dominant plants, with many smaller algae present either on the rock surface or as epiphytes on the stipes of *Laminaria hyperborea*. Table 1 distinguishes the species found in this population. The *L. hyperborea* plants had an average age of 6 ± 2 years, as determined by counting the numbers of growth-rings at the base of the stipe (Parke, unpublished). The maximum age was 8 years. The population extended downwards to a depth of 12 m and appeared to be limited by the absence of continuous rock below this level. Forster (1955) records *L. hyperborea* as extending down to, and disappearing below, 17 m in the vicinity of Stoke Point rocks, Devon.

The vegetation of the floor of the bay

An extensive population, which was more or less loose-lying, was found on the floor of the bay and covering the greater part of its area. The dominant species were *L. saccharina*, *Saccorhiza polyschides*, *Chorda filum* and *Desmarestia aculeata*. The substrates available for attachment in this region consist of sand grains, loose pieces of shell, stones of various sizes, small boulders and other algae. Probably in the first instance most of the algae belonging to this population are attached, perhaps only to sand grains, but at the time that this survey was made, many of them showed no sign of having had an attachment. In the case of *Laminaria saccharina*, the haptera had many branches, but the ends were quite free or intertwined with other algae. Such haptera were, on the whole, smaller than those found for attached plants. Sometimes each hapteron branch was attached to a separate small pebble or piece of broken shell. Many of the smaller algae were found in tangled masses, and in some cases a definite adhesion had been formed so that the plants stuck firmly together. Notable in this respect were the adhesions made by the thalli of *Dictyota dichotoma* and *Plocamium coccineum*, both to other algae and also to stones. Once attached in this way, the fronds of *Dictyota* are able to grow forming flat expanded sheets.

Much of the loose-lying population was at a depth of 10–14 m, but it extended into shallower water. It disappeared, however, where the depth of the

TABLE 1. LIST OF ALGAE FOUND DURING THE SUBLITTORAL SURVEYS 1955 AND 1956

	Percentage occurrence in samples			
	Rock surface population		Loose-lying population	
Species	1955	1956	1955	1956
Laminaria hyperborea (Gunn.) Fosl.	74	72	—	5
L. digitata (Huds.) Lamour.	24	5	4	—
L. saccharina (L.) Lamour.	32	40	96	52
Saccorhiza polyschides (Lightf.) Batt.	40	43	38	30
Chorda filum (L.) Stackh.	2	3	40	37
Desmarestia aculeata (L.) Lamour.	4	12	80	43
Sphacelaria pennata (Huds.) Lyngb.	4	10	62	34
Dictyota dichotoma (Huds.) Lamour.	4	17	60	35
Ulva lactuca L.	26	15	44	25
Phycodrys rubens (Huds.) Batt.	78	63	38	16
Rhodymenia palmata (L.) Grev.	70	60	56	14
Membranoptera altata (Huds.) Stackh.	54	63	10	7
Ceramium rubrum (Huds.) Ag.	42	25	44	16
Ptilota plumosa (Huds.) Ag.	46	33	4	7
Plocamium coccineum (Huds.) Lyngb.	20	25	72	30
Delesseria sanguinea (Huds.) Lamour.	20	7	20	10
Cryptopleura ramosa (Huds.) Kylin	18	53	28	16
Callophyllis laciniata (Huds.) Kütz.	38	17	30	26
Callocolax neglectus Schm.	4	—	2	—
Lithothamnion, etc.	36	27	14	4
Chondrus crispus (L.) Stackh.	14	3	2	6
Cladophora rupestris (L.) Kütz.	10	7	2	8
Sorocarpus micromorus (Bory) Silva	10	3	16	—
Rhodomela confervoides (Huds.) Silva	8	17	30	26
Odonthalia dentata (L.) Lyngb.	8	10	10	8
Brongniartella byssoides (Good. et Woodw.) Schm.	8	12	36	31
Polysiphonia violacea (Roth) Grev.	8	—	16	1
Hypoglossum woodwardii Kütz.	6	7	8	10
Gigartina stellata (Stackh.) Batt.	6	5	10	5
Fucus serratus L.	6	7	4	6
Lomentaria articulata (Huds.) Lyngb.	6	3	6	1
Phyllophora membranifolia (Good. et Woodw.) J. Ag.	6	3	2	—
Apoglossum ruscifolium (Turn.) J. Ag.	6	15	—	—
Gelidium latifolium (Grev.) Born. et Thor.	6	13	—	1
Polysiphonia elongata (Huds.) Harv.	6	—	30	10
P. nigrescens (Huds.) Grev.	6	—	16	6
Corallina officinalis L.	4	5	2	—
Litosiphon pusillus (Carm.) Harv.	4	—	4	10
Plumaria elegans (Bonnem.) Schm.	2	7	2	4
Pterosiphonia complanata (Clem.) Fkbg.	2	3	18	7
Antithamnion sarniense (Lyle) G. Feldm.	2	4	44	—
Chaetomorpha melagonium (Web. et Mohr) Kütz.	3	12	—	2
Fucus vesiculosus L.	2	3	—	2
Halidrys siliquosa (L.) Lyngb.	2	—	—	2
Polysiphonia urceolata (Dillw.) Grev.	2	17	—	4
Ectocarpus confervoides (Roth) Le Jol. s. lat.	2	—	4	1
Cruoria pellita (Lyngb.) Fries	2	—	—	—
Ceramium diaphanum (Lightf.) Roth	2	—	—	—
Stictyosiphon subarticulatus (Aresch.) Reinke	2	—	—	5
Chylocladia verticillata (Lightf.) Bliding	2	—	—	—
Asparagopsis armata Harv., tetrasporophyte	2	—	—	1
Enteromorpha compressa (L.) Grev.	2	—	—	—
E. clathrata (Roth) Grev.	2	—	2	—
Cystoclonium purpureum (Huds.) Batt.	—	25	12	20

TABLE 1 (*cont.*)

	Percentage occurrence in samples			
	Rock surface population		Loose-lying population	
Species	1955	1956	1955	1956
Heterosiphonia plumosa (Ellis) Batt.	—	7	2	1
Cladostephus verticillatus (Lightf.) Ag.	—	3	4	1
Ahnfeltia plicata (Huds.) Fries	—	3	8	7
Alaria esculenta (L.) Grev.	—	7	—	2
Chondria dasyphylla (Woodw.) Ag.	—	5	—	—
Spermothamnion repens (Dillw.) K. Rosenv.	—	3	—	6
Chordaria flagelliformis (Müll.) Ag.	—	3	—	2
Griffithsia flosculosa (Ellis) Batt.	—	3	—	1
Calliblepharis ciliata (Huds.) Kütz.	—	3	—	—
Nitophyllum punctatum (Stackh.) Grev.	—	3	—	—
Bonnemaisonia asparagoides (Woodw.) Ag., sexual plant	—	—	18	7
Gracilaria verrucosa (Huds.) Papenf.	—	—	16	4
Sporochnus pedunculatus (Huds.) Ag.	—	—	16	1
Ceramium tenuissimum (Lyngb.) J. Ag.	—	—	14	1
Bonnemaisonia hamifera Hariot, tetrasporophyte,	—	—	10	1
sexual plant	—	—	2	—
Naccaria wiggii (Turn.) Endl.	—	—	4	—
Polysiphonia nigra (Huds.) Batt.	—	—	4	—
Cutleria multifida (Sm.) Grev.	—	—	2	1
Corynospora pedicellata (Sm.) J. Ag.	—	—	2	—
Arthrocladia villosa (Huds.) Duby	—	—	2	—
Seirospora griffithsiana Harv.	—	—	2	—
Dictyopteris membranacea (Stackh.) Batt.	—	—	2	—
Polysiphonia spiralis Batten	—	—	2	—
Isthmoplea sphaerophora (Carm.) Kjellm.	—	—	2	—
Porphyra umbilicalis (L.) Kütz.	—	—	2	—
Enteromorpha linza (L.) J. Ag.	—	—	2	—
Furcellaria fastigiata (L.) Lamour.	—	—	—	4
Dumontia incrassata (Müll.) Lamour.	—	—	—	2
Spongonema tomentosum (Huds.) Kütz.	—	—	—	2
Asperococcus bullosus Lamour.	—	—	—	2
Scytosiphon lomentaria (Lyngb.) Endl.	—	—	—	1
Myrionema strangulans Grev.	—	—	—	1
Tilopteris mertensii (Sm.) Kütz.	—	—	—	1
Callithamnion tetragonum (Wither.) Ag.	—	—	—	1
Polyides caprinus (Gunn.) Papenf.	—	—	—	1
Holmsella pachyderma (Reinsch) Sturch	—	—	—	1
Chaetopteris plumosa (Lyngb.) Kütz.	—	—	—	1
Halarachnion ligulatum (Woodw.) Kütz.	—	—	—	1
Dudresnaya verticillata (With.) Le Jol.	—	—	—	1
Sphondylothamnion multifidum (Huds.) Näg.	—	—	—	1

The species underlined were found only in the loose-lying population and below 8 m.

water was less than 4 m at low tide. This fact will be referred to again later.

It has not been possible to identify with certainty all the algae found in the loose-lying population; some belong to genera in which the separation of species is at present extremely difficult, while others lack the reproductive stages necessary for identification. Of the eighty-eight species so far identified, fifty-seven were also found growing in the permanent sublittoral population on the rock surface, many were also known to occur in the intertidal region, but eleven were found exclusively in the loose-lying population (see Table 1). The algae belonging to this last group of obligate sublittoral species occurred only in the deeper part of the bay and were not found at a depth of less than 8 m, measured at low tide. Some of the plants found in the loose-lying population, but not in the permanent, are also known to occur in the intertidal region and may have been washed down into the sublittoral, but they appeared quite healthy and many were fruiting. The question as to how far these algae, which were also found in the intertidal region, could be regarded as having developed in the loose-lying population or to have been washed into it during the summer, is not easily answered. Occasional plants of *Laminaria saccharina* showed evidence of having been torn from a rock surface, but the vast majority gave no sign of ever having been attached. It will be shown later that sand grains can give an attachment for algal reproductive bodies sufficient to allow them to segment and develop into new plants. In some cases no further attachment is necessary. In the loose-lying population there was a notable absence of many of the intertidal algae, such as *Ascophyllum nodosum*, *Pelvetia canaliculata* and *Fucus* species, except for *F. serratus* which was present in a very broad form at 4–7 m depth. *Laminaria hyperborea*, which is also often washed up on to the shore in quantity, was absent from the loose-lying population of 1955 and almost so in 1956. Probably owing to the calm weather of 1955, little material had been added from the intertidal region; but, in rougher weather, it seems likely that the latter region would add considerably to the loose-lying population. In this survey, interest centred, not so much in making a species list for the sublittoral algae as in finding out something about their ecological relationships. In April 1955, a dredge haul was taken across Port Erin Bay in a direction which would have passed right through the loose-lying population. On this occasion no algae at all were found. This might have been the result of temporarily unfavourable conditions, the water temperature being very low following two very cold winters and a very cool summer in 1954. In the months following April 1955, there was a period of very calm seas, and sediment settled giving very clear water. The more intense light and warmth of the sunshine passing through the clear water gave ideal conditions for the development of sublittoral algae and an extensive population was found here at the end of August.

The lack of disturbance of the sea bed in this particular year was well illustrated by the form of *Saccorhiza polyschides* growing in this region. On the rock surface in shallower water, the fronds were split into several fingers, but loose-lying plants from a depth of 10 m showed very little splitting of the frond.

It seemed obvious that with any disturbance of the bottom waters, much of the loose-lying population would be washed in and this was observed towards the end of the period of the survey. On 2 September, a south-westerly wind sprang up and waves began to break on the rocks on the north side of the bay. During the following 24 h an appreciable quantity of sea-weed had been washed up beyond the tide line and trials with the grab suggested that some shifting of the central mass had taken place. Attempts to replot the positions of the weed beds, by visual means, failed because sand in the water obscured the view. An enormous quantity of sand was stirred up and its suspension noticeably altered the colour of the water. The heavier particles would settle rapidly, but finer material would continue to restrict the penetration of light to the sea bed for a considerable time. During the next 24 h the wind had again increased and small boulders and stones were washed in with attached weeds.

An analysis of the algae brought in during the first 24 h of the gale showed that it came from three sources: (1) the intertidal region, (2) the sublittoral attached population, (3) the sublittoral loose-lying population. Thus it is seen that under the very favourable conditions of 1955, an extensive, more or less loose-lying population was developed on the floor of Port Erin Bay and that this remained undisturbed through the prolonged calm weather of the summer. As soon as storms came, bringing about movement of the bottom waters, a considerable quantity of weed from this population was carried inshore, but the disturbance failed to remove all of it. At the end of November 1955, there was an appreciable quantity left, but the plants were very broken and rotting. Grab and dredge samples taken at intervals during the winter months showed that many fragments of algae were still present at the end of January. Many of these had begun to proliferate new branches, some of the latter primarily concerned with asexual reproduction, others giving vigorous vegetative growth. Examples of this will be discussed later.

Patches of large boulders give a firm substrate for sublittoral algae in several parts of the bay and these may be conspicuous at all times of the year. One such large patch occurs in the centre of the bay in such a position that a part of it may occasionally just be exposed to the air at extreme low water of spring tides. The distinction between patches of this kind and the general loose-lying population is only one of degree. The stability of any part of the population depends on the size and also on the firmness of the substrate and the degree of water movement.

Naylor (1955), working on species of *Idotea* (Isopoda), found large numbers of these animals feeding on decaying sea-weed in the deep corner of the bay in the angle formed by the south shore and the breakwater. It seems likely, bearing in mind the direction of tidal currents within the bay, that there is a general tendency for the bottom algae to be washed, perhaps very slowly, into this corner where they decay. Grab samples attempted in this position in March 1956 brought up no healthy plants, but many decaying fragments in the mesh of the grab net, and large numbers of *Idotea*.

Conditions in 1956

Unlike the summer of 1955 that of 1956 was stormy and lacking in sunshine. The same general distribution of sublittoral algae was found, but with some significant differences in the second year.

(1) The extent of the loose-lying population was distinctly less than in the previous August and its position was different. Whereas in 1955 the population extended over the major part of the sand of the bay, in 1956 a continuous cover of large algae was only found in shallower water. Elsewhere in the bay algae were present in more scattered patches. Many algae had already been washed up on to the beach and much of the tangled mass in shallow water had probably started life deeper down and been brought inshore by water movement.

(2) There was a considerable difference in the size of the plants of *Saccorhiza polyschides*. In 1955, the majority of the plants found were very large, with well-formed holdfast 'bells' and many with stipes winged and releasing zoospores. In 1956, no mature plants were found; the majority were in the sporeling stage with the bells just beginning to form as small rims on the stipe. The largest bells were little more than half the size of that of an adult plant. It is certain that adult plants, if formed, would have been lost more easily than sporelings, but it is unlikely that none at all would have remained on the floor of the bay. Since no adult plants at all were found in the samples at the end of August, it is likely that the whole population was at an earlier stage of development and this could have been the result of less favourable conditions for growth during this season.

(3) The species composition of the loose-lying population in 1956 was not identical with that of 1955, and many species showed an increase or decrease in quantity between the years. *Gracilaria verrucosa*, *Sporochnus pedunculatus* and *Antithamnion sarniense*, all present in quantity in the deeper parts of the bay in 1955, were quite rare in 1956. *Desmarestia aculeata* also showed a marked decrease in bulk, though it was present in fair quantity in 1956. On the other hand, *Brongniartella byssoides*, found as only occasional plants in 1955, had increased considerably the following year. These differences may have resulted from the deficiencies of the sampling method, but the general impression gained by those working on the survey in both years was that there was a very distinct difference in the species composition, particularly of the loose-lying populations, between the years. At present there is little information concerning the way in which environmental factors affect the individual species, but it seems likely that they do not all react in the same way, and certainly they must influence one another as they thrive or decrease, so that the composition of the population will continually be changing.

Over-wintering of species of the loose-lying population

In considering the question of how the various individual species composing the loose-lying population pass the winter months, one or two points may be noted.

(1) Some of the species do not survive. *Acrothrix gracilis* was dredged in quantity in the bay in 1950, but was not found at all in 1955. One small piece was found again in 1956.

(2) New species may appear. The source of these is at present not certain, but they may result from the practice of the local fishermen of throwing stones and boulders, caught in their nets, into the bay on the inner side of the breakwater. No information is available as to whether species could come from outside the bay as reproductive cells, or as small pieces of thallus travelling in the sea water.

(3) For those species occurring, not only in the loose-lying population, but also in the intertidal and/or in the sublittoral permanent population, e.g. *Laminaria saccharina* and *Saccorhiza polyschides*, plants for the following season could be obtained from these sources. It is interesting, however, that the four larger brown algae which form the bulk of the loose-lying population, *Laminaria saccharina*, *Saccorhiza polyschides*, *Chorda filum*, and *Desmarestia aculeata*, all have the same type of life history, involving an alternation between a large and structurally complex sporophyte and an exceedingly minute gametophyte. Samples of sand and gravel, as well as small stones collected at intervals during the winter months and kept in culture, all yielded young *Laminaria* sporophytes within a few weeks, as did also fragments of algae collected with the stones. This suggests that gametophytes are formed in such large numbers that they cover every available substrate, giving a continuous supply of plants for the loose-lying population.

Fragments of plants of many species were found in samples collected during January and February, many of them in a fruiting condition. The fragments were subjected to culture experiments and stages found in culture were searched for in the field at the same time. This procedure gave information on the way in which some of the individual species pass the winter. A few examples will be given from species for which the evidence is sufficiently complete to form a picture of the overwintering method. Much work remains to be done on these lines before anything like a complete picture can be obtained of the population as a whole.

CULTURE TECHNIQUES

In the culture experiments described below, the plants have been grown in an enriched Erdschreiber solution in glass troughs suspended in constant-temperature culture tanks. The tanks are lighted from above by lamps fixed into movable canopies and both 300 W. filament bulbs, and Atlas Daylight fluorescent tubes are used as alternative light sources. Throughout the experiments the temperature has been maintained at 10° C, and a day length of 18 h has been used. The cultures have been aerated by bubbling air through the culture medium. The medium used has been Erdschreiber (Føyn, 1934) enriched by the addition of 'Tris' (Hydroxymethyl-amino-methane), the disodium salt of Ethylene-diamine-tetra-acetic acid, Vitamin Mix S_3, and the range of trace elements in the concentrations given in the marine medium ASP_2 of Provasoli, McLaughlin & Droop (1957). I am grateful to Dr Provasoli for sending the formula for this medium to me and allowing me to use it before it was published.

A GENERAL CULTURE EXPERIMENT

Six stones each bearing, apparently, only one or two small plants of *L. saccharina* were taken from a dredge sample from the bay in September 1956. The *Laminaria* plants were cut back to a length of about 3 cm above the short stipe, and the stones were kept in culture throughout the winter months, during which time the plants increased to a length ranging from 25 to 30 cm but did not form sporangia. By the beginning of April 1957, the following algae had appeared attached either directly to stones or to the haptera of the *Laminaria* plants: enormous numbers of *Laminaria* sporelings some of which were large enough to be identified as *L. saccharina*, sporelings of *Saccorhiza polyschides*, *Asperococcus bullosus* (unilocular sporangia), *Cladostephus verticillatus*, *Ectocarpus* sp. (plurilocular sporangia), *Ulva lactuca*, *Chaetomorpha melagonium*, *Enteromorpha clathrata*, *Rhodomela confervoides*, *Bonnemaisonia hamifera* (tetrasporophyte), and *Polysiphonia* sp.

Only diatoms appeared on control stones kept in the same culture medium under similar conditions in a second glass trough. If six small stones taken at random from the floor of the bay in this way could produce a population of this kind in culture, presumably from reproductive bodies or juvenile stages already present attached to the stones in the autumn, the source of the sublittoral population for the following year is not far to seek.

It is interesting that *Rhodomela confervoides* appeared on the stones in this culture experiment. This alga is found frequently in the intertidal region and was plentiful in the sublittoral loose-lying population during the summers of both 1955 and 1956. It was found also in dredge samples in January 1956 as unattached broken fronds which had lost all secondary branches and were so ragged in appearance as to be hardly recognizable. Arising from the denuded main axes, however, were large numbers of very short erect branches ending in dense tufts producing tetrasporangia. Almost frondless midribs of *Delesseria sanguinea* producing tetrasporic branches in enormous numbers were also found in the same dredge sample and it is possible that other red algae behave in a similar manner in the deeper waters of the bay during the winter. If algae are able to reproduce freely in this way in the sublittoral, this region may be extremely important in acting as a source of new populations for the intertidal region also. Much more detailed work, however, would be required to establish this as an ecological principle.

INDIVIDUAL SPECIES

Laminaria saccharina

The life history of *L. saccharina* includes two distinct morphological phases, a structurally elaborate diploid plant alternating with an extremely minute gametophyte, the latter consisting of a branched filament of a few cells, sometimes reduced to a single cell (Williams, 1921; Harries, 1932). Parke (1948) reported, as a result of her investigations, that 'sporophytes of *Laminaria saccharina* can be produced in nature during all months of the year at some level of the shore,.... The gametophyte generation of the species must, therefore, be capable of reproduction during all months of the year'. In the sublittoral zone, 1–4 m below E.L.W.S.T., Parke reports cessation of sporophyte development from November to February. She suggests that this is due to low light intensity. Parke gives the region in the sublittoral zone of 6–12 m below E.L.W.S.T. as a habitat in which no development of young plants was obtained on prepared surfaces. She remarks, however, that well developed plants of *L. saccharina* have been brought up from depths down to 12 m and suggests that, since these were growing on shingle, they may have been washed down into deeper water, having started their development in shallower water.

Sporelings of *L. saccharina* have been found in samples taken from the floor of Port Erin Bay during all months of the year. There seems little doubt that sporophyte production occurred on quite a large scale at depths down to 14 m here during the winter of 1955–56. A dredge sample taken across the bay at the end of January 1956 included the following: (1) plants of *L. saccharina* with the previous year's frondage attached to new frondage. The plants had either no apparent attachment or they were attached to stones and shells. Some had obviously been torn away from a rock surface, but had new hapteron branches attached to small stones, suggesting that this latter attachment had developed while the fronds were lying on the bottom gravel; (2) very small plants attached to stones and other algae; (3) broken pieces of large plants.

Both whole fronds and also broken pieces were found with ripe sporangia. Zoospores were released and allowed to settle in sea water on glass slides in glass dishes which were kept covered in a dim light in a constant temperature room at 10° C. Later the dishes were transferred to the constant temperature culture tank at 10° C, again under a low light intensity. The zoospores germinated to give both male and female gametophytes. In the female gametophytes no branched filaments were formed. The development was similar to that described by Williams (1912) and Harries (1932) as sometimes occurring in this species. After the zoospore had settled, a germination tube grew out and the contents of the spores passed into its swollen end which was then cut off by a wall. The swollen cell, which Harries described as the 'effective plant' functioned directly as an oogonium. Harries's work suggested that this might have been the effect of the culture conditions in which a fairly low temperature and a low light intensity were used, a high temperature and light intensity inducing a filamentous form in the gametophyte. Although no idea has yet been obtained of the exact conditions at the bottom of the bay, the temperature and light levels during the winter months would certainly have been below those used in the culture experiments. Fertilization was not observed, but since male gametophytes producing antheridia were also present on the same slides, it may actually have occurred. Since, however, young sporophytes were observed to be developing before any antheridia were seen, there is a possibility that parthenogenesis may have occurred. This has been recorded for *L. saccharina* by Schreiber (1930), though his haploid sporophytes showed an abnormal development. Since the zoospore can act directly as an oogonium and the male gametophytes are exceedingly small, a particle as small as a sand grain would be sufficient to provide attachment for the growth of the new sporophyte. Zoospores are produced in enormous numbers by the mature sporophytes so that, given suitable conditions of temperature and light intensity, and if undisturbed too greatly by water movements, there is the potentiality for the production of a large sublittoral population of *L. saccharina*. That this does, in fact, happen was shown by the presence, in samples taken at the end of March by dredge, of large numbers of sporelings of varying sizes, attached to sand grains and to small and large stones and to other algae. The range of size of frond found suggested that sporophyte production had proceeded throughout the winter. That a proportion of the sporophytes, even including some of very small size, had started to develop the previous autumn, was shown by a distinction in the fronds between narrow and broad regions of growth. A period of more active vegetative growth is reported by Parke (1948) to begin in January and the autumn and spring regions of growth can be distinguished by a marked difference in width in the frond. Since the young sporophytes were found in dredge samples, the exact depth from which they were taken could not be determined, but their presence on one or two large boulders taken in a position close to the seaward end of the breakwater suggests that they can develop at a depth approaching 14 m.

In culture the developing sporophytes were removed from the slides as soon as they had reached a size of between 0·5 and 1 cm and were large enough to handle and they were left lying loose in the culture solution. They continued to develop, and by May 1957 had reached a maximum length of 30 cm and a width of 8 cm. The total amount of frondage formed during this period was, however, greater than is indicated in this length measurement since decayed frondage was cast at the free end, while new tissue was added from the meristem above the stipe. It is of particular interest that the haptera of these plants have developed many branches and appear in all respects similar to those found on plants in the loose-lying population suspected of having developed unattached on the floor of the bay (Fig. 1). It appears therefore that, except in the very earliest stages of the development of the sporophyte, an attachment to a substrate is not necessary for the growth of *L. saccharina*. That the free hapteron branches can become secondarily attached to a substrate is suggested by systems of the type in which each branch is attached separately to a small stone or shell.

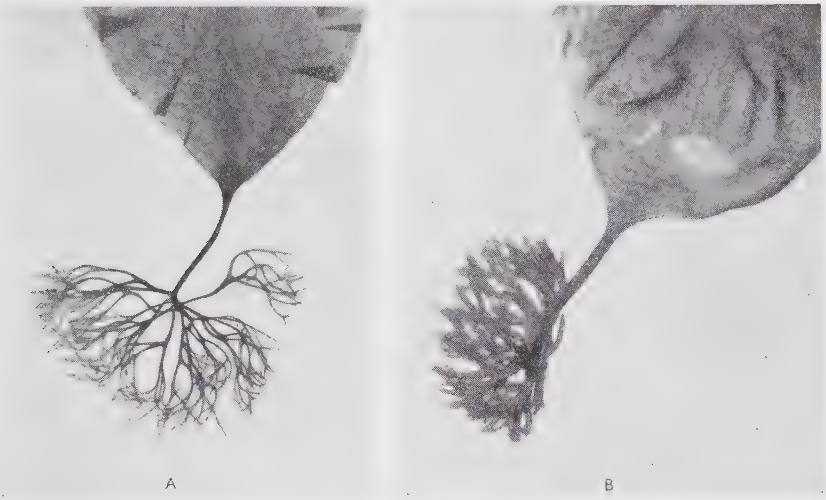

Fig. 1. Hapteron systems of *Laminaria saccharina* (L.) Lamour. A, plant from loose-lying population (from herbarium sheet) $\times \frac{2}{9}$; B, plant grown unattached in culture $\times \frac{2}{3}$. Photos J. H. Bloor.

Saccorhiza polyschides

Spence (1918) reports that for the Orkney Islands, during the winter months the fronds of *S. polyschides* are washed ashore, but that the attaching 'bells' which become very firmly attached to the substrate, remain behind. Possibly this also happens in the sublittoral permanent population in Port Erin Bay, but it was not possible to find them. Samples taken in the winter months here showed that, while the majority of the mature plants, together with the stipes carrying the reproductive frills, had disappeared, there were still many basal bells lying loose on the floor of the bay. Some of these were dredged in January 1956, dark with sporangia all over the surface of the wart-like projections. Zoospores were released from these and allowed to settle on slides which were kept in culture in the laboratory. Male gametophytes were formed as short slender branched filaments, but, as with *Laminaria saccharina*, there was a tendency for the female gametophyte to be represented by a single cell: the first division cut off a cell which behaved as an oogonium developing directly into a new sporophyte. Fertilization was not observed. By March the sporophytes had reached a length of 3–4 cm. Sampling by dredge in Port Erin in March produced similar sporophytes in large numbers on stones and other algae. Some of the sporophytes found in the field were larger than those grown in culture, but many were at the same stage of development. Probably for *Saccorhiza polyschides* the attaching bell is of greater importance as a reproductive structure than the frill to the stipe which disappears earlier in the autumn with the blade of the plant. Such loose-lying bells, rolling round the floor of the bay with any water movement and releasing zoospores in large numbers, could easily give rise to the population for the ensuing year.

Desmarestia aculeata

D. aculeata occurred as large plants in both rock surface and loose-lying populations. This plant was not found in the fertile condition and its behaviour in culture could not be followed. Its behaviour was, however, followed by field observations. In March 1956 many plants and even small fragments of plants were taken from the bay which were regenerating by the production of new lateral branches. This type of regeneration has been observed by Söderström (1889) and Printz (1926). The new branches could readily be distinguished from those of the previous season by the tufts of delicate lateral branches which later disappear. Attempts to produce these new vegetative branches in culture were not successful.

Sporelings were also obtained from dredge samples in March: these were found attached to shells, to small stones and to sand grains. Schreiber (1932) has shown that the zoospores released by adult fronds give rise to filamentous gametophytes bearing oogonia and antheridia; and it may be assumed that these are formed on stones and shells and sand grains on the sea floor, sporelings later arising from them.

This plant therefore appears to have two distinct methods of building up its numbers for the following year, first, by regeneration from old plants and fragments of plants and, secondly, by the completion of its dimorphic life-history at the bottom of the bay.

DISCUSSION

To determine the behaviour of the sublittoral loose-lying population as a whole, one must know the behaviour of its component species and the interrelationships between them, and autecological studies will have to be made on the majority of its species. The composition of the population undoubtedly changes from time to time, both in the identity of the component species and also in their relative abundance. Changes in the conditions to which the population is subjected will almost certainly affect different species in different ways and the species themselves will affect one another by their absence or presence and abundance. Since the sublittoral loose-lying population has many species in common with that of the sublittoral rock-surface and intertidal areas, it is likely that there are complex interactions between the three regions. A slight indication that this is so is given by the fact that some species, as for example *Rhodomela confervoides*, occurring frequently in the intertidal region, can reproduce freely in the deeper parts of the bay during the winter months. The evidence given in this paper suggests that a detailed knowledge of the requirements and behaviour of algae in the sublittoral region will help towards an understanding of the ecology, not only of this, but also of the intertidal region.

I am much indebted to the Council of the Royal Society for a grant from the Browne Research Fund towards the cost of the survey, and I wish to thank Prof. N. A. Burges for help and encouragement throughout the course of the work. The work was carried out from the Marine Biological Station in Port Erin, and I should like to thank the Director, Mr J. S. Colman and his Staff, particularly Mr A. B. Bowers and Dr J. Kain, for their help in many ways: also Mr J. Faragher and Mr L. Collister, the crew of the 'Cypris'. The spring grab was loaned by the Institute of Seaweed Research, Musselburgh, by kind permission of the Director, Dr F. N. Woodward and I should also like to thank Mr F. T. Walker for much help and advice on the use of the grab for this survey work. Dr P. S. Dixon was responsible for the echo-sounding of the bay and Mr N. Hawkins of the Liverpool Sub-Aqua Club made the dives to check the grab. The following helped with the field work either in 1955 or 1956 or in both years: Mr B. Appleby, Miss M. Beaumont, Mr J. Chubb, Mrs P. S. Dixon, Miss J. Hawkins, Mr B. Kendrick, Mr I. Prestt and Mr G. Strafford. My thanks are due to all of these people for their help.

SUMMARY

A combination of dredge, grab and diving techniques was used to make a survey of the sublittoral algae of Port Erin Bay, Isle of Man. Two distinct populations were found, one attached to the rock surface on the sides of the bay and the other more or less loose-lying on its sandy floor.

The loose-lying population is conspicuous only during the summer months, usually from July until October, and is washed up on to the beach by storms or decays on the floor of the bay.

Culture experiments combined with field observations at different times of the year have been used to determine the overwintering methods of some of the individual species of the loose-lying population. For two of the dominant species, *Laminaria saccharina* and *Saccorhiza polyschides*, both of which are

extreme dimorphic diplohaplonts, it has been shown that gametophytes are formed in extremely large numbers on all possible substrates on the floor of the bay during the winter and give a continuous supply of young sporophytes to renew the population.

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OBSERVATIONS ON LUMINESCENCE IN PELAGIC ANIMALS

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(Plate I and Text-figs. 1–19)

Luminescence is very common among marine animals, and many species possess highly developed photophores or light-emitting organs. It is probable, therefore, that luminescence plays an important part in the economy of their lives. A few determinations of the spectral composition and intensity of light emitted by marine animals are available (Coblentz & Hughes, 1926; Eymers & van Schouwenburg, 1937; Clarke & Backus, 1956; Kampa & Boden, 1957; Nicol, 1957*b*, *c*, 1958*a*, *b*). More data of this kind are desirable in order to estimate the visual efficiency of luminescence, distances at which luminescence can be perceived, the contribution it makes to general background illumination, etc. With such information it should be possible to discuss more profitably such biological problems as the role of luminescence in intraspecific signalling, sex recognition, swarming, and attraction or repulsion between species. As a contribution to this field I have measured the intensities of light emitted by some pelagic species of animals.

Most of the work to be described in this paper was carried out during cruises of R. V. 'Sarsia' and R.R.S. 'Discovery II' (Marine Biological Association of the United Kingdom and National Institute of Oceanography, respectively). Collections were made at various stations in the East Atlantic between 30° N. and 48° N. The apparatus for measuring light intensities was calibrated ashore at the Plymouth Laboratory; measurements of animal light were made at sea. In addition to the physical measurements, some histological descriptions of the light organs of several species are presented, together with miscellaneous observations on regulation of flashing in several species.

MATERIALS AND METHODS

Animals were captured in vertical and oblique hauls with a 2 m stramin net or ring trawl (R.T.), or with an Isaacs Kidd trawl (I.K.T.); and in surface hauls with a 1 m stramin net (N. 100).

Stations were as follows.

'Discovery' stations:

No. 3344. 18 Oct. 1955. 41° 16′–17½′ N., 9° 28′ W. R.T. oblique. 1000 m of wire out, estimated depth 500 m.

No. 3354. 6 Nov. 1955. 31° 12·5′ N., 15° 58′ W. to 31° 14·5′ N., 16° 01·5′ W. R.T. oblique; estimated depth 250 m.
No. 3355. 8 Nov. 1955. 30° 13′ N., 15° 58′ W. R.T. surface.
No. 3365. 18 Feb. 1956. 43° 47·2′ N., 13° 8·6′ W. R.T. oblique. 1500 m of wire out; estimated depth about 1000 m.
No. 3366. 19 Feb. 1956. 41° 11′ N., 14° 35′ W. R.T. oblique. 2000 m of wire out; estimated depth about 1300 m.
No. 3368. 21 Feb. 1956. 41° 03′ N., 14° 23′ W. R.T. oblique. 2000 m of wire out; estimated depth about 1300 m.
No. 3369. 24 Feb. 1956. 38° 39′ N., 9° 40·5′ W. N. 100 surface.
No. 3372. 28 Feb. 1956. 41° 09′ N., 14° 35′ W. R.T. oblique. 3000 m of wire out; estimated depth about 2000 m.
No. 3374. 29 Feb. 1956. 41° 11′ N., 14° 34′ W. R.T. oblique. 3000 m of wire out; estimated depth about 2000 m.
No. 3375. 2 Mar. 1956. 41° 09′ N., 14° 33′ W. R.T. oblique. 3000 m of wire out; estimated depth about 2000 m.
No. 3376. 6 Mar. 1956. 46° 59·5′ N., 7° 54·5′ W. R.T. oblique. 3000 m of wire out; estimated depth about 2000 m.

'Sarsia' stations:

No. 1, cruise 6/55. 17 June 1955. 46° 49′ N., 5° 44′ W. R.T. vertical. 280 m of wire out.
No. 5, cruise 3/57. 20 June 1957. 46° 55′ N., 5° 57′ W. I.K.T. oblique. 1800 m of wire out; estimated depth about 1200 m.
No. 6, cruise 3/57. 20 June 1957. 47° 07′ N., 6° 06′ W. R.T. vertical haul. 1829 m of wire out.
No. 8, cruise 3/57. 20/21 June 1957. 47° 00′ N., 6° 05′ W. Hand nets. Surface.
No. 10, cruise 3/57. 21 June 1957. 46° 57′ N., 6° 09′ W. I.K.T. oblique. 1800 m of wire out; estimated depth about 1200.
No. 21, cruise 3/57. 28 June 1957. 46° 47′ N., 5° 56′ W. R.T. oblique. 2742 m of wire out; estimated depth about 1800 m.
No. 23, cruise 3/57. 29 June 1957. 46° 42′ N., 6° 22′ W. I.K.T. oblique. 1800 m of wire out; estimated depth 1000 m.

The animals were examined either immediately on capture, or they were retained in cold sea water in a refrigerator for several hours. Some measurements were made of animals at room temperature (> 14° C), but it was found that animals from deep waters survived better when placed in cooled sea water. Accordingly, an arrangement was devised for delivering cooled sea water to the vessel containing the animal, and this arrangement was used for deep-sea species. The method consisted of taking sea water from the ship's system, and passing it through a lead coil lying in a refrigerator. A visual indicator was placed in the circulation to monitor flow-rate. Water temperatures were noted for each experiment, and are given in the text.

Measurements of spectral composition and intensity were made by means of a multiplier phototube (E.M.I. type no. 6685). The photomultiplier was connected to a cathode-ray oscilloscope having a d.c. amplifier. Mains supply for the photomultiplier and oscilloscope was regulated by a voltage stabilizer.

Occasionally two oscilloscopes were used, having different amplifications. Deflexions on the oscilloscope screen were photographed on moving paper. A time scale was provided by a periodically flashing light, regulated by a Palmer relay.

The spectral sensitivity of the photomultiplier (type no. 6685) had been determined by the National Physical Laboratory; a curve is given elsewhere (Nicol, 1958*a*, fig. 2A). The photomultiplier was calibrated against a sub-standard lamp (supplied by the National Physical Laboratory). From the data a constant, q, was calculated, such that $q/S_\lambda = \mathrm{W/cm^2}$ of a given wavelength (λ) required to produce unit deflexion

$$q = \frac{p}{D_L}\int_{400}^{700} \mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda \ \mathrm{W/cm^2} \text{ receptor surface,}$$

$$= 0{\cdot}49 \times 10^{-12} \ \mathrm{W/cm^2} \text{ receptor surface.}$$

S_λ = relative sensitivity of photomultiplier E.M.I. type no. 6685 at a given wavelength; $\mathcal{J}_\lambda$ is the spectral energy distribution of the substandard lamp; T_λ is the transmission of neutral filters used in the measurements; D_L is the deflexion occasioned by the substandard lamp on the cathode-ray oscilloscope at a given amplification; and p is a quantity for the particular experimental set-up. The method and calculations are described in more detail elsewhere (Nicol, 1958*a*).

For daily checks on the sensitivity of the apparatus at sea, a stable light source was employed, which consisted of a stilbene phosphor irradiated by ^{60}Co (supplied by the United Kingdom Atomic Energy Authority). This source had a measured output of 0·07 μlm.

Spectral sensitivity was determined by a method making use of a rotating disc containing alternating spectral and reference filters about its circumference. Spectral filters were Ilford gelatine and Chance glass. Reference filters were Ilford blue-green 603 or Ilford green 604. Two discs were used, containing the filters listed in Table 1, in order of rotation. Disc I was intended for analysing green light, Disc II for blue light. Curves for the combined effect of filter transmission (T_λ) and photomultiplier sensitivity (S_λ) are given in Text-figs. 1 and 2 (S_λ T_λ plotted against λ). The luminescent flashes vary greatly in intensity, and the responses obtained with the reference filters gave periodical values for fluctuating flash intensity. First approximate results for relative spectral composition of luminescent light were corrected for wide band-width of the filters. The method is described in more detail in earlier papers (Nicol, 1957*b*, *c*).

When measuring intensity, the animal was placed at a known distance directly beneath the photomultiplier. When measuring spectral emission, the rotating disc was aligned close beneath the photocathode, so that each filter in turn passed across the face of the latter. The animal was placed as close as possible to the disc, vertically underneath the end window of the photomultiplier (see Nicol, 1957*c*, fig. 1). The species from which luminescence was recorded are listed in Table 2.

For studying regulation of luminescence, controlled electrical stimulation was employed, either condenser shocks or square wave pulses. Electrodes were Ag, Pt, sometimes non-polarizable Ag/AgCl. Some earlier recordings were made with an R.C.A. photomultiplier, type no. 931A, with battery supply; extracts of these records have already been published (Nicol, 1955*a*). Later observations were made with E.M.I. photomultiplier, type no. 6685.

Particulars concerning chemistry and histology are given in the text.

TABLE 1. FILTERS IN ORDER OF ROTATION

Disc I	Disc II
Double space (position marker)	Double space (position marker)
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
Ilford red 608	Ilford orange 607
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
Ilford orange 607	Ilford yellow 606
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
Ilford yellow 606	Ilford yellow-green 605
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
Ilford yellow green 605	Ilford blue-green 603 + neutral density D 0·5
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
Ilford green 604 + neutral density D 0·5	Ilford blue 602 + neutral density D 0·5
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
Ilford blue 602 + neutral density D 0·5	Ilford violet 601
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
Ilford violet 601	Chance purple OV 1
Ilford blue-green 603 + neutral density D 0·5	Ilford green 604
	Chance u.v. OX 1

OBSERVATIONS AND RESULTS

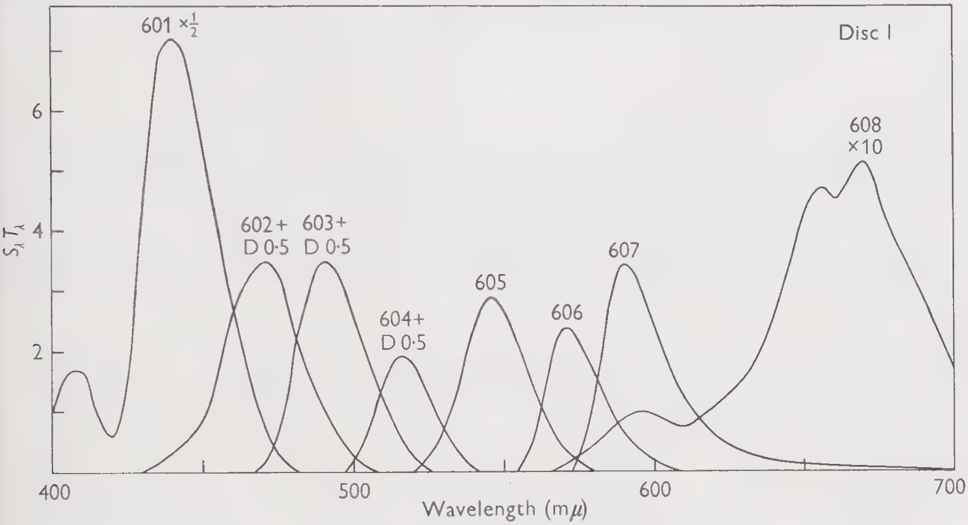
In order to calculate radiant flux, a curve for relative spectral emission is required (E_λ plotted against λ). This curve is then put on an absolute basis in terms of a quantity r, such that $E_\lambda r$ gives W/cm² of receptor surface/mμ under the experimental conditions specified.

$$r = \frac{Dq}{E_\lambda S_\lambda \mathrm{d}\lambda} \ \mathrm{W/cm^2/m\mu},$$

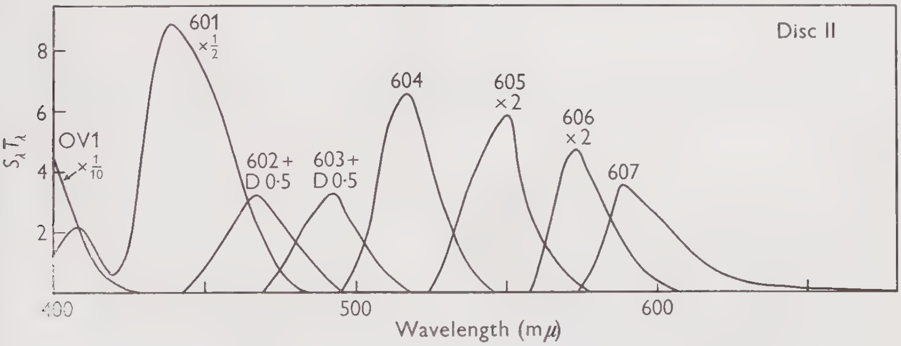
$$r = D \times \frac{0{\cdot}49 \times 10^{12}}{E_\lambda S_\lambda \mathrm{d}\lambda} \ \mathrm{W/cm^2/m\mu}.$$

Data for various species, and calculated values of light intensities, are assembled in Table 2. Details are presented in the following sections, arranged according to zoological classification.

Radiolaria. Two specimens were examined belonging to the species *Cytocladus major* Schröder and *Aulosphaera triodon* Haeckel ('Discovery' Sta. no. 3375). Both specimens were spherical in shape and about the same diameter (some 12 mm). That they were two separate species was not apparent at the time, and the records of their luminescence were not distinguished from each other. Results for both specimens are combined.



Text-fig. 1. Curves showing the combined effect of sensitivity of the photomultiplier (S_λ) times transmission of spectral filters (T_λ) used in recording Disc I ($S_\lambda T_\lambda$ plotted against λ). Neutral filters, having a density of 0·5, were used in conjunction with Ilford spectral filters, nos. 602, 603 and 604.



Text-fig. 2. Curves showing the combined effect of sensitivity of the photomultiplier (S_λ) times transmission of spectral filters (T_λ) used in recording Disc II ($S_\lambda T_\lambda$ plotted against λ). Neutral filters, having a density of 0·5, were used in conjunction with Ilford spectral filters, nos. 602 and 603.

TABLE 2. CALCULATIONS OF THE INTENSITY OF LIGHT EMITTED BY SOME PELAGIC ANIMALS

Group and species	Response, mm (deflexion, D_s)	Response duration (sec)	Response averaged over 1 sec (D)	Recording distance (L) in air (cm)	Radiant flux, μW or μJ/cm² receptor surface at distance L	Temp. (° C)	Size of animal
Radiolaria							
Cytocladus major and	5·78	2·7	3·9	5·6	$1{\cdot}70 \times 10^{-6}$ μW	22	1·2 cm diam.
Aulosphaera triodon	0·82	1·1	0·4	5·6	$0{\cdot}18 \times 10^{-6}$ μW	22	1·2 cm diam.
	1·45	1·7	0·8	5·6	$0{\cdot}35 \times 10^{-6}$ μW	22	1·2 cm diam.
Hydromedusae							
Colobonema sericeum	2·6	1·9	2·1	9·7	$1{\cdot}01 \times 10^{-6}$ μW	8	4 cm diam
	1·9	1·9	1·6	9·7	$0{\cdot}77 \times 10^{-6}$ μW	8	4 cm diam.
Crossota alba	0·8	*ca.* 1	0·05	13	$0{\cdot}02 \times 10^{-6}$ μW	13	2·5 cm diam.
Aeginura grimaldii	1·48	3·8	0·99	14	$0{\cdot}47 \times 10^{-6}$ μW	11	*ca.* 1·2 cm diam.
Siphonophora							
Vogtia spinosa	7·98	11·2	7·58	14	$3{\cdot}48 \times 10^{-6}$ μW	8	2 cm diam.
	1·94	1·9	1·49	14	$0{\cdot}68 \times 10^{-6}$ μW	8	2 cm diam.
	4·08	3·1	2·45	14	$1{\cdot}12 \times 10^{-6}$ μW	8	2 cm diam.
	19·35	3·8	11·61	14	$5{\cdot}32 \times 10^{-6}$ μW	12	2 cm diam.
	13·3	3·3	10·9	17·4	$5{\cdot}00 \times 10^{-6}$ μW	21·8	1 cm diam.
	29	2·9	23·2	17·4	$10{\cdot}64 \times 10^{-6}$ μW	21·8	1 cm diam.
	14·52	2·9	10·60	17·4	$4{\cdot}86 \times 10^{-6}$ μW	21·8	1 cm. diam.
V. glabra	72·80	3·5	42·2	7·8	$19{\cdot}35 \times 10^{-6}$ μW	8	2 cm diam.
Rosacea plicata	6·3	1·9	2·6	10·7	$1{\cdot}19 \times 10^{-6}$ μW	20	2.5 cm diam.
	2·4	1	1·2	7	$0{\cdot}55 \times 10^{-6}$ μW	20	2·5 cm diam.
Hippopodius hippopus	1·22	2	0·93	8	$0{\cdot}43 \times 10^{-6}$ μW	22	0·8 × 0·3 cm
	2·99	1	1·50	7·7	$0{\cdot}69 \times 10^{-6}$ μW	22	1·3 cm diam.
Scyphomedusae							
Atolla wyvillei	28·1	1·6	21·4	14	$10{\cdot}24 \times 10^{-6}$ μW	13	9 cm diam.
(and one *A. parua*)	19·3	1·4	8·27	14	$3{\cdot}96 \times 10^{-6}$ μW	13	9 cm diam.
	1·25	0·14	0·29	14	$0{\cdot}14 \times 10^{-6}$ μW	13	9 cm diam.
	1·3	0·6	0·35	8·9	$0{\cdot}17 \times 10^{-6}$ μJ	24	5 cm diam.
	0·54	0·5	0·27	8·9	$0{\cdot}13 \times 10^{-6}$ μJ	24	5 cm diam.
	0·35	1·0	0·20	5·7	$0{\cdot}10 \times 10^{-6}$ μW	24	1·2 cm diam.
	3·08	1·1	1·54	9·0	$0{\cdot}74 \times 10^{-6}$ μW	9	2·7 cm diam.
	2·96	1·9	1·48	9·0	$0{\cdot}71 \times 10^{-6}$ μW	9	2·7 cm diam.
	1·13	1·3	0·79	9·0	$0{\cdot}38 \times 10^{-6}$ μW	9	2·7 cm diam.
	3·42	1·9	2·50	9·0	$1{\cdot}20 \times 10^{-6}$ μW	9	2·7 cm diam.
	2·84	1·4	1·87	9·0	$0{\cdot}90 \times 10^{-6}$ μW	9	2·7 cm diam.
	2·27	1·9	1·25	9·0	$0{\cdot}60 \times 10^{-6}$ μW	9	2·7 cm diam.

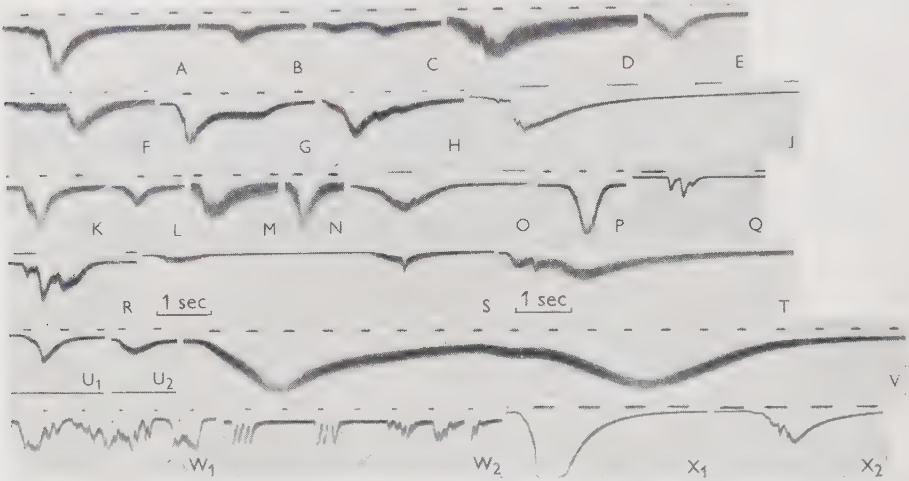
Ctenophora							
Beroë ovata	29·3	0·8	17·6	28·4	10·42 × 10^{-6} μJ	14	1 × 1·2 cm
	11·8	0·8	3·9	28·2	2·31 × 10^{-6} μJ	11	1 × 1·2 cm
	70·2	0·18	28·8	26	17·05 × 10^{-6} μJ	24·5	1 × 1·5 cm
	83·6	0·6	27·8	26·9	16·46 × 10^{-6} μJ	24·5	1 × 1·5 cm
	6·7	0·1	2·5	24·4	1·48 × 10^{-6} μJ	24·5	1 × 1·5 cm
	53·6*	1	16·4*	34·4	9·71 × 10^{-6} μW	24	1 × 1·5 cm
	300*	1·6	200*	26·9	118·4 × 10^{-6} μW	24·5	1 × 1·5 cm
	66·3	0·5	26·5	26·9	15·69 × 10^{-6} μJ	24·5	1 × 1·5 cm
	13·8	0·7	6·9	26·9	4·08 × 10^{-6} μJ	24·5	1 × 1·5 cm
	34·4	0·8	22·4	27·4	13·26 × 10^{-6} μJ	24	1 × 1·5 cm
	4·6	0·3	1·5	13·8	0·89 × 10^{-6} μJ	24	2 × 4 cm
	13·8	0·3	1·9	13·8	1·12 × 10^{-6} μJ	24	2 × 4 cm
Crustacea							
Acanthephyra purpurea	0·9	1	0·5	9	0·23 × 10^{-6} μW	9	—
	2·9	1·4	1·0	9	0·46 × 10^{-6} μW	9	—
	2·4	4·7	2·2	9	1·01 × 10^{-6} μW	9	—
Tunicata							
Pyrosoma atlanticum	2·6	4·7	2·5	10·8	1·18 × 10^{-6} μW	14	1 × 4 cm
	10·8	2·3	4·8	20·8	2·3 × 10^{-6} μW	24·5	1·2 × 6 cm
	5·5	1·7	3·6	20·8	1·7 × 10^{-6} μW	24·5	1·2 × 6 cm
	8·3	11	7·9	27	3·7 × 10^{-6} μW	20	1 × 4 cm
	35·4	17	35	28·3	17 × 10^{-6} μW	23	0·9–1·2 × 4·4 cm
Teleostei							
Myctophum punctatum	0·56	0·9	0·20	9·5	0·09 × 10^{-6} μJ	16	88 mm long
	11·97	2·0	7·06	9·5	3·20 × 10^{-6} μW	16	88 mm long
	1·03	1	0·51	9·5	0·23 × 10^{-6} μW	16	85 mm long
	1·88	1	0·47	9·5	0·21 × 10^{-6} μW	16	85 mm long
	1·71	0·42	0·42	9·5	0·19 × 10^{-6} μJ	16	89 mm long
	25·65	4·23	12·83	9·5	5·81 × 10^{-6} μW	16	89 mm long
Searsia schnakenbecki	57	2	36	9	19 × 10^{-6} μW	11	12·7 cm long†
	150	2·3	100	9	53 × 10^{-6} μW	11	12·7 cm long†
	55		37	9	20 × 10^{-6} μW	11	12·7 cm long†
S. koefoedi	321	3·9	245	14·7	130 × 10^{-6} μW	12–15	12·2 cm long†
	245	4	184	14·7	98 × 10^{-6} μW	12–15	12·2 cm long†

* Deflexion off screen. Rough estimate.

† Standard length.

Both radiolarians gave a luminescent glow, lasting some 1–2 sec when subjected to tactile stimulation (Text-fig. 3 A, B, C). The light had a bluish colour and it illuminated the whole sphere, although it appeared brightest at the centre. Neither of these two species has hitherto been reported to be luminescent (cf. Harvey, 1952). The light of other radiolarians is blue and of low intensity (Brandt, 1885; Harvey, 1955).

Light intensity of *Cytocladus major* and *Aulosphaera triodon*, measured at a distance of 5·6 cm in air, ranged from $0{\cdot}2\times10^{-6}$ to $1{\cdot}7\times10^{-6}\,\mu\mathrm{W/cm}^2$



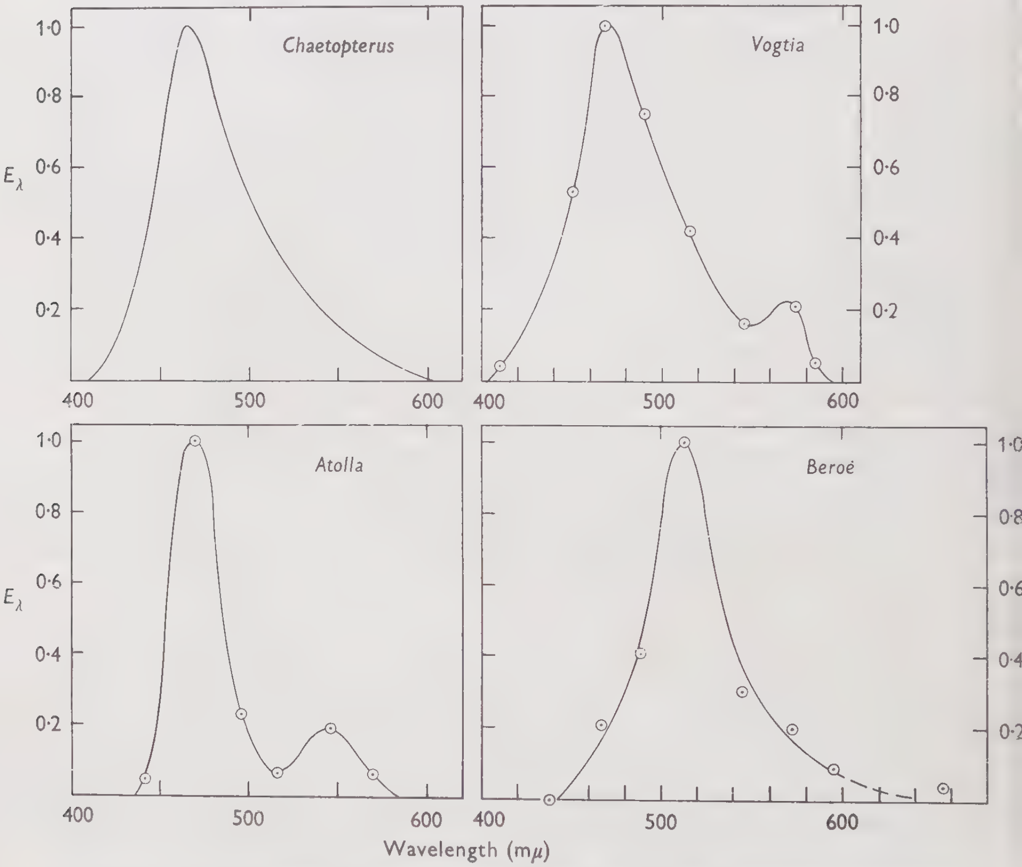
Text-fig. 3. Oscillograph records of the luminescent responses of pelagic animals. Deflexion downwards of the oscilloscope beam corresponds to the luminescent glow or flash. Periodical time scale above. A–C. Flashes of radiolarians (*Cytocladus major* and *Aulosphaera triodon*), evoked by mechanical stimulation. Water temperature, 22° C. Time scale, period 0·96 sec. D. *Aeginura grimaldii*. Glow evoked by mechanical stimulation. Temperature of sea water 11° C. Time scale, period 0·97 sec. E, F. Responses of *Colobonema sericeum* to mechanical stimulation. Time, 1/sec. Temperature of sea water 8° C. G, H. Flashing of *Vogtia spinosa* under mechanical stimulation. Time scale, period 0·98 sec. Temperatures of sea water: G, 12° C, H, 21·8° C. J. *Vogtia glabra*. Luminescence evoked by mechanical stimulation. Time scale, 1/sec. Temperature of sea water 8° C. K, L. Luminescence of *Rosacea plicata* evoked by mechanical stimulation. Time scale, period 0·96 sec. Temperature of sea water 20° C. M, N. *Hippopodius hippopus* (Forskål). Luminescent responses resulting from mechanical stimuli. Time scale, 1/sec. Water temperature about 22° C. O, P. Flashes of *Atolla wyvillei* under mechanical stimulation. Time, 1/sec. Water temperature 11° C. Q, R. Flashes of *Beroë ovata* subjected to mechanical stimulation. Time scale, period 0·96 sec. Temperature of sea water, 24° C (Q), 11° C (R). S, T. 'Puffs' of light produced by the luminous discharge of a deep-sea shrimp, *Acanthephyra purpurea*. Luminescence was provoked by tactile stimulation. Time scale, 1 sec. Temperature of sea water, 9° C. Amplification of S 0·58 times that of T. U_1, U_2. *Pyrosoma atlanticum*. Glow produced by mechanical stimulation of whole animal (6 cm long). Time, 1/sec. Water temperature, 24·5° C. V. *Pyrosoma atlanticum*. Glow following mechanical stimuli (animal 4 cm long). Time period, 0·96 sec. Temperature of sea water, 20° C. W_1, W_2. *Myctophum punctatum*. Flashing of photophores induced by mechanical stimulation of intact living fish. Time scale, 1/sec. Water temperature, 16° C. X_1, X_2. *Searsia koefoedi*. Records of the glow produced when a fish discharges a luminous secretion into sea water. Tactile stimulus. Time scale, 1/sec. Water temperature 12–15° C.

TABLE 3. INTENSITY OF LUMINESCENCE OF SOME PELAGIC ANIMALS

Group and species	Radiant flux, μW or μJ/cm² receptor surface			Temp. (° C)	Source
	Measured Flux	Recording distance (cm)	Recalculated Flux at 1 m distance in air		
Radiolaria					
Cytocladus major and	0·2 × 10^{-6} μW	5·6	0·6 × 10^{-9} μW	22	Original
Aulosphaera triodon	1·7 × 10^{-6} μW	5·6	5·3 × 10^{-9} μW	22	Original
Hydromedusae					
Colobonema sericeum	0·8 × 10^{-6} μW	9·7	7·2 × 10^{-9} μW	8	Original
	1·0 × 10^{-6} μW	9·7	9·5 × 10^{-9} μW	8	Original
Crossota alba	0·02 × 10^{-6} μW	13	0·4 × 10^{-9} μW	13	Original
Aeginura grimaldii	0·5 × 10^{-6} μW	14	9·3 × 10^{-9} μW	11	Original
Siphonophora					
Vogtia spinosa	0·7 × 10^{-6} μW	14	13·7 × 10^{-9} μW	8	Original
	10·6 × 10^{-6} μW	17·4	320·9 × 10^{-9} μW	21·8	Original
V. glabra	19·4 × 10^{-6} μW	7·8	120 × 10^{-9} μW	8	Original
Rosacea plicata	0·6 × 10^{-6} μW	7	2·4 × 10^{-9} μW	20	Original
	1·2 × 10^{-6} μW	10·7	13·7 × 10^{-9} μW	20	Original
Hippopodius hippopus	0·4 × 10^{-6} μW	8	2·6 × 10^{-9} μW	22	Original
	0·7 × 10^{-6} μW	7·7	4·2 × 10^{-9} μW	22	Original
Scyphomedusae					
Atolla wyvillei	0·1 × 10^{-6} μW	5·7	0·3 × 10^{-9} μW	24	Original
	10·2 × 10^{-6} μW	14	199·9 × 10^{-9} μW	13	Original
Ctenophora					
Beroë ovata	0·89 × 10^{-6} μJ	13·8	16·95 × 10^{-9} μW	24	Original
	118 × 10^{-6} μW	26·9	8538·5 × 10^{-9} μW	24·5	Original
Mnemiopsis leidyi	0·5 × 10^{-4} μW	50	12,500 × 10^{-9} μW	—	Clarke & Backus, 1956
	> 0·75 × 10^{-4} μW	50	18,750 × 10^{-9} μW	—	Clarke & Backus, 1956
Crustacea					
Acanthephyra purpurea	0·23 × 10^{-6} μW	9	1·9 × 10^{-9} μW	9	Original
	1·01 × 10^{-6} μW	9	8·2 × 10^{-9} μW	9	Original
Euphausia pacifica	1·6 × 10^{-3}–2 × 10^{-3} μW	1	160 × 10^{-9}–200 × 10^{-9} μW	—	Kampa & Boden, 1957
Tunicata					
Pyrosoma atlanticum	1·18 × 10^{-6} μW	10·8	13·8 × 10^{-9} μW	14	Original
	17 × 10^{-6} μW	28·3	1361·5 × 10^{-9} μW	23	Original
	8 × 10^{-3}–4 × 10^{-2} μW	1	800 × 10^{-9}–4000 × 10^{-9} μW	—	Kampa & Boden, 1957
Teleostei					
Searsia schnakenbecki	19 × 10^{-6} μW	9	150 × 10^{-9} μW	11	Original
	53 × 10^{-6} μW	9	430 × 10^{-9} μW	11	Original
S. koefoedi	98 × 10^{-6} μW	14·7	2117 × 10^{-9} μW	12–15	Original
	130 × 10^{-6} μW	14·7	2808 × 10^{-9} μW	12–15	Original
Myctophum punctatum	0·1 × 10^{-6} μJ	9·5	0·925 × 10^{-9} μJ	16	Original
	5·8 × 10^{-6} μW	9·5	52·345 × 10^{-9} μW	16	Original

receptor surface (Table 2). By assuming that the light intensity falls off with distance according to the inverse square law, values can be calculated for light intensities at a standard distance of 1 m in air, viz. $0{\cdot}55 \times 10^{-9}$ to $5{\cdot}34 \times 10^{-9}$ μW/cm^2 receptor surface (Table 3). Deviation from the inverse square law, owing to size of the source, decreases with distance. In the present instance, the error from this factor is small, and the flux calculated at 1 m may be considered a minimal estimate. The luminescence of these radiolarians appeared somewhat diffuse. If they were uniformly diffusing spheres, the total radiant flux from the entire surface of the animals would range from $0{\cdot}69 \times 10^{-4}$ to $6{\cdot}71 \times 10^{-4}$ μW/4π steradians.

No spectral emission curve is available for the light of radiolarians, and in the above calculations I have employed the spectral curve for *Chaetopterus* light as a substitute.



Text-fig. 4. Relative spectral emission curves for the light of four marine animals, namely, *Chaetopterus variopedatus*, *Vogtia glabra*, *Atolla wyvillei* and *Beroë ovata*.

Chaetopterus light is blue, and has a peak at about 465 mμ (Text-fig. 4) (Nicol, 1957*c*). Since the response curve of the photomultiplier is fairly flat below 500 mμ (Nicol, 1958*a*, fig. 2A), a difference of 10 or 20 mμ in the maximum of the spectral emission curve used in making the calculations would result in only a small change in estimated flux. I have calculated radiant flux on the basis of a spectral emission curve resembling that in Text-fig. 4, but displaced 20 mμ towards longer wavelengths; the result for the first response given in Table 2 is $2 \cdot 02 \times 10^{-6}$ μW/cm^2 receptor surface at 5·6 cm in air. This differs from the response given in the table ($1 \cdot 70 \times 10^{-6}$ μW) by 19 %.

Cytocladus is widespread in the Atlantic and *C. major* has been found previously in a catch from 3000 m (Schröder, 1907). *Aulosphaera triodon* appears to be cosmopolitan (Haecker, 1907).

HYDROMEDUSAE

Crossota alba Bigelow. A single specimen was collected ('Discovery' Sta. no. 3376). The jellyfish was bell-shaped, and about 24 mm in diameter. When mechanically stimulated it gave a very weak bluish glow lasting about 1 sec. It soon fatigued, for a second and further stimuli evoked much weaker responses. The spectral emission curve for *Atolla* luminescence (see p. 718) was used to calculate radiant flux. *A. wyvillei* also emits a blue light, extending from about 410 to 600 mμ, with a peak at 470 mμ (Text-fig. 4, p. 714). According to Harvey (1952), the light of two other Hydromedusae (*Aequorea* and *Mitrocoma*), examined with a hand spectroscope, extends from about 460 to 600 mμ. At 1 m in air, estimated radiant flux for *Crossota alba* is $0 \cdot 4 \times 10^{-9}$ μW/cm^2 receptor surface.

This is the first report of luminescence in *C. alba*. It is a deep-water jellyfish known previously from the North Pacific and tropical Atlantic (Kramp, 1947, 1957).

Aeginura grimaldii Maas. Two specimens of this jellyfish were examined ('Discovery' Sta. no. 3376). With light mechanical stimulation they emitted a bluish glow lasting 1–4 sec (Text-fig. 3D). An estimate of light intensity was made, the calculation being based on the relative spectral emission curve for *Atolla* light (Text-fig. 4). At a distance of 1 m (in air), minimal estimated radiant flux is $9 \cdot 3 \times 10^{-9}$ μW/cm^2 receptor surface. *A. grimaldii* is a bathypelagic species of world-wide distribution (Russell, 1953), in which luminescence has not previously been reported.

Octophialucium funeraria (Quoy & Gaimard). This is a deep-sea medusa, reaching a diameter of about 4 cm. One specimen was examined ('Sarsia' Sta. no. 1, cruise 6/55). When mechanically stimulated by gently stroking it with a camel-hair brush, it emitted a bluish glow. No measurement of light intensity was made. Its luminescence is here noted, since it seems not to have been previously reported. *O. funeraria* has been found at various stations in the Atlantic and Mediterranean.

Colobonema sericeum Vanhöffen. One specimen was examined ('Sarsia' Sta. no. 5, cruise 3/57). This medusa is bell-shaped, up to 4 cm in diameter and height. It is a deep-water species, found in temperate and warm regions of the Atlantic, Pacific and Indian Oceans (Russell, 1953). When mechanically stimulated, the specimen of *C. sericeum* emitted a blue glow, lasting about 2 sec (Text-fig. 3E, F). A spectral emission curve was not obtained, and that for *Atolla* (Text-fig. 4) was used in calculating light intensity. At a distance of 1 m (in air), estimated radiant flux would be 7×10^{-9} to 9×10^{-9} μW/cm^2 receptor surface. This appears to be the first report of luminescence in *Colobonema sericeum*.

Other luminescent species of Hydromedusae are noted by Russell (1953). Certain physiological and histological observations on luminescence and photogenic tissue of *Aequorea* have been presented by Davenport and Nicol (1955).

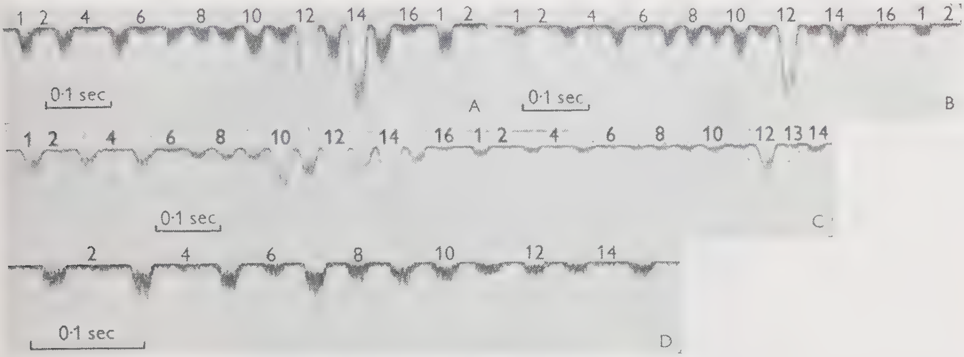
SIPHONOPHORA

Vogtia spinosa Keferstein & Ehlers. Specimens were obtained from 'Discovery' Sta. nos. 3374 and 3376. When examined in the dark, fragments of this species were found to be markedly luminescent. Light, evoked by mechanical stimulation, appeared as a bluish glow diffused through the fragment (nectophores or gastrozooids). The specimens appeared very sensitive to mechanical stimuli, and responded by glowing to roll of the ship, and slight tapping of the vessel in which they lay. Luminescence was not inhibited by exposure to light (60 W tungsten lamp at about 1 m). The glow lasts from 2 to 11 sec, depending on the strength of stimulus (Text-fig. 3G, H). To calculate light intensity, a spectral emission curve obtained for *V. glabra* was used (Text-fig. 4). At 1 m (in air), minimal estimates of radiant flux range from 14×10^{-9} to 321×10^{-9} μW/cm^2 receptor surface. This appears to be the first report of luminescence in *V. spinosa*, which is a widely distributed bathypelagic species known from the Atlantic, Pacific, and Mediterranean (Bigelow & Sears, 1937).

V. glabra Bigelow. A fragmented specimen was obtained ('Sarsia' Sta. no. 21, cruise 3/57). Fragments emitted a bluish luminescence under mechanical stimulation. The glow lasted some 4 sec (Text-fig. 3J). An approximate spectral emission curve was obtained with the aid of Disc II. This was spun at speeds of 1–2 rev./sec. The specimen was positioned close beneath the disc, and luminescence was evoked by gentle tactile stimulation (Text-fig. 5). Water temperature was 8° C. Spectral emission extends from about 430 to 590 mμ, with a maximum at about 470 mμ (Text-fig. 4). A single measurement of light intensity afforded an estimate of 120×10^{-9} μW/cm^2 at 1 m (in air) (Table 3). *V. glabra* is a bathypelagic species, found in the Mediterranean and the Atlantic (Leloup, 1955), and known to be luminescent (see Totton, 1954).

Rosacea plicata Bigelow. Fragments of a specimen were obtained ('Discovery'

Sta. no 3374). Luminescence was evoked by mechanical stimulation. The light had a blue hue, and the responses lasted 1–2 sec (Text-fig. 3 K, L). The spectral emission curve of *V. glabra* (Text-fig. 4) was used in calculating light intensity. At 1 m (in air), estimated radiant flux is $2{\cdot}4\times10^{-9}$ to $13{\cdot}7\times10^{-9}$ μW/cm^2 receptor surface (Table 3). *R. plicata* has a world-wide distribution. Although usually obtained in catches from deeper waters, it has occasionally been found at the surface (Bigelow & Sears, 1937; Leloup, 1955).



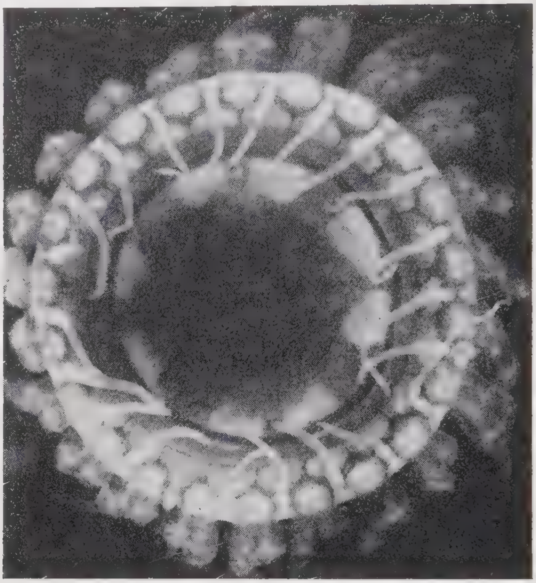
Text-fig. 5. A, B. Oscilloscope deflexions obtained when Disc II was used to analyse the light of *Vogtia glabra*. Time scale, 0·1 sec. Filters in order: 1, 604; 2, OX 1; 3, 604; 4, 607; 5, 604; 6, 606; 7, 604, 8, 605; 9, 604; 10, 603; 11, 604; 12, 602; 13, 604; 14, 601; 15, 604; 16, OV 1. Temperature of sea water 8° C. Disc speed, *ca.* 1·7 rev./sec. C. Analysis of the light of *Atolla wyvillei* with Disc II. Numbering of filters as in A and B. Water temperature 20° C. Speed of rotation about 1·7 rev./sec. Time, 0·1 sec. D. Analysis of the light of *Beroë ovata* with Disc I. Filters in order: 1, 603; 2, 608; 3, 603; 4, 607; 5, 603; 6, 606; 7, 603; 8, 605; 9, 603; 10, 604; 11, 603; 12, 602; 13, 603; 14, 601; 15, 603. Temperature of sea water, about 19° C. Disc speed about 1 rev./sec.

Hippopodius hippopus (Forskål). Fragments of a specimen were obtained ('Discovery' Sta. no. 3369). When mechanically stimulated the animal emitted a blue light lasting 1–2 sec (Text-fig. 3 M, N). The spectral emission curve for the light of *Vogtia glabra* (Text-fig. 4) was used for calculating radiant flux. At 1 m (in air), estimated flux was $2{\cdot}6\times10^{-9}$ to $4{\cdot}2\times10^{-9}$ μW/cm^2 receptor surface. There are previous reports of luminescence in this species (Dubois, 1914; Totton, 1954). *Hoppopodius hippopus* occurs in the Mediterranean and in warmer regions of the three oceans (Totton, 1954; Leloup, 1955).

SCYPHOMEDUSAE

Atolla wyvillei Haeckel. Many specimens were examined from stations as follows: 'Discovery' Sta. nos. 3366, 3368, 3372, 3374, 3375 and 3376; 'Sarsia' Sta. no. 21, cruise 3/57. These jellyfish are umbrella-shaped, ranging up to 15 cm in diameter. When mechanically stimulated, some specimens emitted a faint bluish light all around the margin; some emitted only a weak point of light in the immediate vicinity of stimulation; still others remained dark. These differences in behaviour possibly depend upon the condition of the specimen, amount of buffeting it has received in the net, etc. Luminescence has not hitherto been reported in this species.

Light appears as a brief flash or glow, lasting up to 2 sec (Text-fig. 3 O, P). Relative spectral composition of the light was determined by means of recording Disc II (Text-fig. 5C). The light is blue in colour, and emission occurs at about 470 mμ, with hint of a secondary peak at about 570 mμ. Calculated values of light intensity are given in Tables 2 and 3. At a distance of 1 m (in air), minimal estimates of the intensity of *Atolla* luminescence range up to 200×10^{-9} μW/cm^2 receptor surface.



Text-fig. 6. Dorsal view of *Atolla wyvillei*. From a colour transparency of a living animal.

When a specimen of *A. wyvillei* was subjected to carefully localized mechanical stimulation with a probe, the light appeared in a thin streak just inside the peripheral edge of the thick coronal muscle, at the base of the rhopalar peduncle, between two tentacles (Text-fig. 6). It was visible from the upper surface, but not from the lower surface of the jellyfish. The thick coronal muscle of *Atolla* is opaque and white. The photogenic tissue must be above this muscle layer, which prevents the light from shining through to the lower surface. The white muscle band may also act as a reflector for the luminescence.

The white opacity of the coronal muscle extends throughout the thickness of the latter. The white appearance is not destroyed by the following agents: water, formalin, ethanol, ether, 1 and 10% acetic acid, 1% HCl, 10% NH_3, and 0·1% NaOH. These negative results rule out the possibility that the white appearance is due to some deposit of purine material such as guanine, pterine, or even uric acid. It is probably a characteristic of the physical structure of the muscle or its contained connective tissue.

Histological sections were cut from the edge of the umbrella to search for luminescent tissue. The material was fixed in formalin, and post-fixed in Wittmaack's solution. Sections were cut in celloidin, and stained with Weigert's haematoxylin, Bordeaux red and aniline blue. A patch of tall columnar epithelial cells occurs in the floor of the rhopalar canal, and in its connexion with the canal running along the lateral wall of the rhopalar pedalium, opposite the nematocyst cushion. These columnar cells constitute a modified endodermal lining, which elsewhere in the canal consists of cuboidal cells. They have a very glandular appearance. The nucleus is almost central; the basal half of the cell is vacuolar, whereas the distal half is packed with small granules. The latter are stained mostly blue (with aniline blue), but some larger granules have taken up the Bordeaux red preferentially. Near the distal margin of each cell is a small spherical clear area, appearing like a lenticular organelle. Cellular dimensions are: height, about 45 μ; width, 12 μ; peripheral clear area, about 9 μ in diameter. The position and glandular character of these cells suggest that they may represent the luminous tissue.

A. wyvillei is a bathypelagic species having a cosmopolitan distribution (Kramp, 1947).

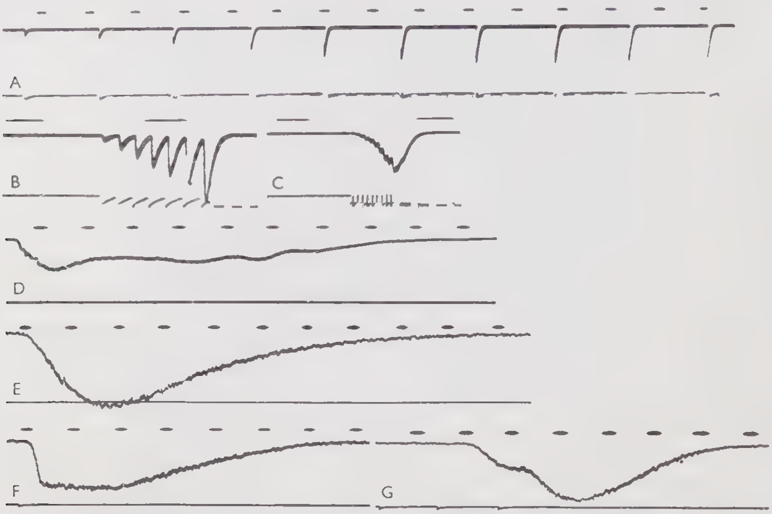
Among the specimens of *Atolla* in which luminescence was observed was one of *A. parva*, the new species recently described by Russell (1958).

Periphylla periphylla (Péron & Lesueur). Two specimens of this jellyfish were examined and were found to be luminescent ('Sarsia' Sta. no. 1, cruise 6/55). When mechanically stimulated they emitted a blue glow around the margin, either in the tentacles, or in the rim of the umbrella. The light was not recorded. There appears to be no previous notice of its luminescence. *P. periphylla* is bathypelagic and has a world-wide distribution (Kramp, 1947).

CTENOPHORA

Beroë ovata Bosc. (= *B. cucumis* Fabricius). Specimens were examined from 'Discovery' Sta. nos. 3366, 3368 and 3376; and from 'Sarsia' Sta. no. 5, cruise 3/57. The light of *Beroë* appears green to the eye (cf. Harvey, 1955). A few records, obtained with Disc I, gave enough data to make an approximate estimation of spectral composition (Text-fig. 5D). A strip of the animal, containing several combs, was pinned out in a black dish underneath the disc, and light was evoked by stimulating the preparation with a camel-hair brush. On analysing the results, it appears that light emission extends from about 440 to 650 mμ, with a peak around 510 mμ (Text-fig. 4, p. 714).

The relative spectral composition curve, shown in Text-fig. 4, was used to calculate light intensities. At a distance of 1 m (in air), estimated radiant flux is $1{\cdot}7 \times 10^{-8}$ to $8{\cdot}5 \times 10^{-6}$ μW/cm^2 receptor surface (Tables 2 and 3). Clarke & Backus (1956), using a different method, have measured the intensity of light emitted by the ctenophore *Mnemiopsis leidyi*. At 50 cm distance, radiant fluxes were $0{\cdot}5 \times 10^{-4}$ and at least $0{\cdot}75 \times 10^{-4}$ μW/cm^2 receptor surface (two determinations). When calculated for a distance of 1 m (in air), the equivalent intensities are $0{\cdot}1 \times 10^{-4}$ and $> 0{\cdot}2 \times 10^{-4}$ μW/cm^2 (Table 3).



Text-fig. 7. A, B, C. Flashes of *Beroë ovata* under electrical stimulation. Point responses. Stimuli intervals: A, 1·24 sec; B, 0·11 sec; C, 0·029 sec. Time scale above, 1/sec. Stimuli on lower line. Water temperature 20–21° C. D–G. Responses of *Pyrosoma atlanticum* to tactile and mechanical stimuli. D. Tactile stimulation. Local response and transmitted waves. Specimen 10 cm. long. Time scale, 0·98 sec. Water temperature 25° C. E. Local response to tactile stimulus. Time scale, 0·96 sec. Water temperature 25° C. F. Local response to an electric shock (shown on lower line). Water temperature 25° C. Time, 0·96 sec. G. Responses to burst of shocks at 1/sec. Water temperature, 24° C. Time, 1/sec. A low-pass filter was used when recording traces D–G to reduce noise.

When *Beroë* is mechanically stimulated, waves of luminescence run along the meridional combs. The conducting system is non-polarized, since transmission occurs with facility in either direction (Panceri, 1872). The same responses occur in *Mnemiopsis* (see Moore, 1924). The light appears in two closely spaced thin green lines along each of the combs, seemingly associated with paired strips of luminous tissue in the walls of the meridional canals (see Harvey, 1952). Under strong mechanical stimulation, the light has a flickering character, owing to repetitive flashing occurring along the length of the comb, such flashes continuing when stimulation has ceased. Multiple or repetitive flashing occurs also in *Beroë* under electrical stimulation (Nicol, 1955*a*), and it has been observed likewise in *Mnemiopsis* (see Chang, 1954).

The luminescence of ctenophores is inhibited by light, and specimens of *Beroë* were dark-adapted before use. Pieces containing one or several combs were pinned out on wax and were covered with sea water. Electrical stimuli were delivered through wire electrodes placed on the combs.

With local responses, i.e. those confined to the immediate vicinity of the electrodes, it is found that each shock above threshold produces a flash (Text-fig. 7A). With repetitive stimulation, the consecutive flashes increase progressively in intensity for some time (perhaps 8–12 flashes), and then reach maximum or plateau level, after which decline sets in. The increment of consecutive flashes is best seen when stimulation is at a rate slow enough for each response-record to return to base line, and for no summation of the light of successive flashes (Text-fig. 7A). In records of transmitted waves of light, obtained from long lengths of comb, the intensity relationships of single flashes are obscured by repetitive responses. At fast rates of stimulation (9/sec or more), the individual flashes tend to fuse together but they can still be distinguished at frequencies of 34/sec (Text-fig. 7B, C).

Under repetitive stimulation, augmentation of intensity can be produced by two factors, summation of light when the flashes recur at brief intervals, less than the duration of a single flash, and facilitation occasioned by build-up of an excitatory state such that one pulse leaves a residual condition enhancing second and later responses. Although the effect is somewhat irregular, there is a tendency for facilitatory increment to be greater at faster rates of stimulation (or shorter intervals), as shown in Text-fig. 8. The effect was also brought out as follows. Paired pulses were given at selected intervals and the responses measured. The ratios of the second to the first flash were:

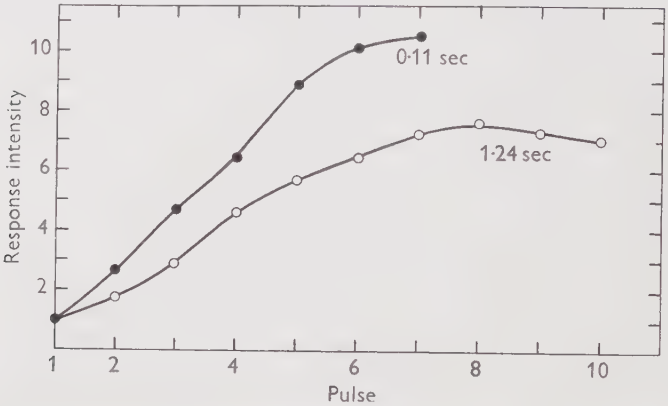
Interval between flashes (sec)	Ratio of intensity of second to first flash
120	1·3
60	1·2
12	1·4
4	2
1·68	2
1·24	1·8
0·62	1·8
0·24	2
0·11	2·7

Some residual facilitation is still detectable 2 min after a previous pulse. A similar increment of facilitation with rise in frequency appears to hold in *Mnemiopsis* (see Chang, 1954, p. 382, fig. 9). Continued stimulation leads to falling off in intensity, i.e. to fatigue. This becomes apparent after 10 or so pulses. A single flash, from a point area, has a duration of about 0·16 sec (20–21° C). Latent period is about 20 msec.

The progressive increase in intensity of consecutive flashes, shown in Text-fig. 7A, is one more manifestation of neuro-effector facilitation, so well documented in muscular responses of sea anemones and other coelenterates

(Pantin, 1952). It has also been detected in the luminous responses of hydromedusans and pennatulids (Davenport & Nicol, 1955; Nicol, 1955*b*). It is not known where the facilitatory process occurs in ctenophores, but there is evidence in polynoids that it can occur within the photocytes or light-generating cells (Nicol, 1957*a*).

Beroë ovata is a cosmopolitan form, found usually in upper waters (Chun, 1880; Bigelow, 1912; Mayer, 1912; Mortensen, 1912; Krumbach, 1927; Sears, 1954).



Text-fig. 8. Facilitatory increment in consecutive flashes of *Beroë ovata* at two frequencies (intervals shown). Ordinates show relative intensities. Temp. 20–21° C.

CRUSTACEA DECAPODA

Acanthephyra purpurea M.-Edw. Luminescence was observed in a deep-sea shrimp belonging to this species ('Sarsia' Sta. no. 5, cruise 3/57). This specimen appears to be *A. purpurea* sensu stricto, since it has only four pairs of dorso-lateral spines on the telson, and no tooth on the dorsal carina of the fourth abdominal segment (Kemp, 1939). *A. purpurea* is a widely distributed bathypelagic shrimp found in the Atlantic, south of 50° N., in the Pacific and in the Indian Oceans (Kemp, 1910, 1939; Stephenson, 1923; Balss, 1925).

Examined in the laboratory in darkness, *Acanthephyra* was found to discharge a luminous secretion from the head region into the surrounding sea water when the animal was excited mechanically. The light had a blue colour, and the discharge continued to glow for 1–5 sec (Text-fig. 3 S, T). Luminescence was soon exhausted under repeated stimulation, and it was not possible to determine spectral composition.

Since the light of *A. purpurea* has a bluish colour, I have used the relative spectral emission curve of *Cypridina* light, as determined by Coblentz & Hughes (1926), for calculating light intensity. *C. hilgendorfii* also discharges a luminous secretion and its light is blue in colour, with maximal emission at about 480 mμ (Text-fig. 19A). The intensity of the discharge ranged from 2×10^{-9} to 8×10^{-9} μW/cm^2 receptor surface at a distance of 1 m (in air).

The discharge of a luminous secretion from the antennal glands or head glands of various other species has been observed repeatedly, e.g. in *Heterocarpus alphonsi*, *Plesiopennaeus* (= *Aristaeus*) *coruscans*, *Hoplophorus novaezealandiae*, *H. spinicauda* and *Systellaspis debilis*. The last-named is a species that also possesses integumentary photophores (Alcock, 1902; Dahlgren, 1916; Chace, 1940; Dennell, 1940, 1955; Harvey, 1952.)

TUNICATA

Pyrosoma. Specimens for observation and histology were collected as follows. *P. atlanticum* Péron—'Discovery' Sta. nos. 3344, 3354, 3355, 3365, 3366, 3374 and 3375; 'Sarsia' Sta. no. 23, cruise 3/57; one specimen from deep water off San Diego, California, 12 December 1953. *P. spinosum* Herdman—'Sarsia' Sta. no. 1, cruise 6/55.

Pyrosoma is one of the most brightly luminescent animals of the open ocean. Its light emission was first noted by Péron in 1804, and has been commented upon repeatedly—see descriptions by Bennett (1840), Huxley (1851), Moseley (1892), and Steuer (1910); and reviews of the literature by Neumann (1934), and Harvey (1952).

Pyrosoma is a colonial animal and light is emitted by each of the zooids. During a normal response an excited colony appears aglow from numerous points of blue light. Before presenting some original observations I would point out that the luminescence of *Pyrosoma* shows a combination of peculiarities not found in other animals. First there is a general agreement that the light of *Pyrosoma* is intermittent. Secondly, the light is transmitted as a wave along the colony, although nervous connexions between the zooids still await discovery. Thirdly, luminescence is evoked by illumination. Fourthly, the light is ascribed to luminescent symbiotic bacteria. This belief, fostered by Buchner (1914) and Pierantoni (1921), now appears to be generally accepted (Brien, 1948; Young, 1950; Caullery, 1952). Other luminescent bacteria, however, emit light continuously (discussion in Harvey, 1952).

Pyrosoma luminesces only when excited, and effective stimuli are mechanical, electrical, photic and chemical. The light appears in a punctate pattern over the whole surface; at each locus two closely spaced points of light can be resolved, which correspond to the pair of light organs at the entrance to the branchial sac of each zooid (*P. atlanticum*, cf. Panceri, 1873). In addition, there is a diffuse glow, the result of scattering and internal reflexion within the colony.

Light tactile stimulation of a fresh specimen produces a luminous wave, which spreads outwards from the stimulated region to the limits of the cylinder. A strong mechanical stimulus, such as squeezing the animal, causes the whole surface to lighten. Some recordings of the light emitted under mechanical stimulation are shown in Text-fig. 3 (U_1, U_2, V) (p. 712) and Text-fig. 7D.

With repeated tactile stimulation, the luminescent response becomes progressively weaker, transmission finally ceases, and the light is confined to the region of stimulation (Text-fig. 7E).

It has long been known that luminescence in *Pyrosoma* can be induced by illumination and a *Pyrosoma* in the dark will respond to the flash of another *Pyrosoma* by luminescing in turn. When a weak light from a torch is shone upon a colony, it responds by flashing (Nicol, 1955*a*, p. 312, fig. 9). The response to weak illumination appears to be of the same character as that induced by tactile stimulation; it begins with a local glow which spreads as a wave over the colony. An instance was observed in which the light, after proceeding from one end of the colony to the other, gradually retreated to the end first illuminated, where the light outlasted the response elsewhere.

The luminescent flashes of *Pyrosoma* are rather slow and prolonged compared with those of many other luminescent animals. The following measurements for *P. atlanticum* are given as examples.

Specimen 1 cm long. Tactile stimulation evoked transmitted luminous waves. Duration of response 13·8 sec. Time to maximum 1·06 sec; time to ½ maximum 0·41 sec; decay time 12·8 sec; ½ decay time 1·39 sec. Most of the response was finished (½ decay) by 2·5 sec (temp. 25·5° C).

Specimen 10 cm long. Tactile stimulation produced transmitted luminous waves. Time to maximum from first deflexion, 0·4–1·6 sec. For a response lasting 8½ sec, time to maximum lasted 1·6 sec; ½ maximum was reached in 1 sec; time for ½ decay was 2·9 sec (temp. 25·5° C).

Specimen 3 cm long. Local responses to electrical stimulation. Latency varied from 0·1 to 0·4 sec. In a response lasting 7·2 sec, maximum intensity was reached in 0·3 sec; ½ maximum in 0·15 sec; ½ decay from maximal deflexion occupied 3·3 sec (temp. 25·5° C).

For *P. elegans* (*P. atlanticum*), Polimanti (1911) found that the response varied from 5 to 30 sec (average 10 sec). Latencies varied from 1 to 5 sec; the average for photic stimuli was 5 sec. With very weak photic stimuli, the latency was prolonged; in general, the latency was shorter with stronger stimuli.

Nerves to the photogenic organs have not been detected, and it has been claimed that they are not innervated (Julin, 1912; Neumann, 1934). It is possible, of course, that the nerve fibres may be very fine, and may have been overlooked with the methods employed. The colonies are very sensitive to mechanical stimulation and, in the absence of innervation, would be acting as independent effectors. Following brief photic stimulation, transmission of luminescence across a colony can take place as a chain reaction, the light from one zooid stimulating its neighbours, and so on until the light wave has crossed the colony. As Burghause (1914) has shown, such transmission can occur even in the absence of nervous connexions. Pyrosomas often occur at the surface in shoals, and it seems likely that when one animal is excited, its light may cause other colonies nearby to luminesce.

For histological examination specimens of *P. atlanticum* and *P. spinosum* were fixed in various solutions, including formalin, Bouin's, Helly's, Heidenhain-Susa, 80% ethanol, and osmic acid. Sections cut in paraffin wax and celloidin were stained with Ehrlich's haematoxylin and eosin, Biebrich scarlet and erythrosin, Heidenhain's haematoxylin and van Gieson, Masson's trichrome, Heidenhain-azan, Giemsa and Bodian's activated protargol.

In *P. atlanticum* the photogenic organs lie on each side of the pharynx above the peripharyngeal bands. They consist of a mass of photogenic cells, one or several tiers in thickness, lying in the peripharyngeal blood sinus. The cells are closely spaced, cell boundaries are sometimes distinguishable, but in many places the limits of separate cells cannot be resolved (Pl. I). In shape they tend to be spherical, about 9–15 μ in diameter. In *P. atlanticum*, subsp. *giganteum*, cell size is given as 25–30 μ (Julin, 1912). The nucleus is peripheral, containing closely packed granular material; the cytoplasm is densely packed with inclusions, the presence of which is correlated with light production, and which constitute the photogenic paraplasm.

The paraplasmic inclusions are tubular structures, some 2 μ in thickness, containing fine particles or granules. The tubules appear curved, like half-rings, but they may actually, as Julin (1912, 1913) states, form a convoluted thread, only segments of which appear in focus at one time. This point is rather difficult to resolve. They stain with acid stains, eosin, erythrosin, Biebrich scarlet, orange G; diffusely and irregularly with haematoxylin; and with both xylidine ponceau and light green of Masson's trichrome stain in a spotty manner. In sections treated with Giemsa the inclusions are stained by the methylene blue. Their affinity for dyes is rather poor and they need to be overstained to confer much colour on them. Their structure is probably revealed as well, or better, by Bodian's silver proteinate, than by any of the more orthodox acidic dyes of the azo and xanthene groups. They are not dissimilar to the illustrations of Julin (1912), although much more densely packed than shown by him.

The photocytes of *P. spinosum* resemble those of *P. atlanticum*.

Pierantoni (1921) claimed that the inclusions were symbiotic bacteria, and he described and illustrated dividing bodies and spores inside the photocytes. I have not seen such reproductive bodies and spores in the photogenic cells of my material. It seems to me that dense areas and apparent nodes in the cytoplasmic inclusions can be ascribed to overlapping of inclusions, or end-views of the inclusions which are seen vertically rather than in profile. Julin (1912) suggested that the inclusions contained nuclein (i.e. nucleoprotein), since internal granulations stained with haematoxylin. To test this possibility, sections were treated with Feulgen's reagent for deoxyribose nucleic acid. The test was negative: the photogenic inclusions were not coloured. Therefore, I can offer no additional evidence for the existence of symbiotic light bacteria.

According to Julin (1912), ontogenetically the photogenic organs are formed from test cells, which are transmitted from one generation to the next. These test cells accompany the egg, and pass from cyathozooid to primary ascidiozooids. The test cells or follicular cells of the eggs of *Pyrosoma* are also luminous (Julin, 1913). In this developmental process, the photogenic inclusions (symbiotic bodies of Pierantoni) are supposed to be transmitted from the parent ascidiozooids to the luminous organs which develop in the ascidiozooids of the larval colonies (Buchner, 1914, 1953; Pierantoni, 1914). Development and budding are described by Berrill (1950*b*).

Measurements of light intensity were made from several specimens of *P. atlanticum*, and the results are presented in Tables 2 and 3 (pp. 711 and 713). For relative spectral composition I have used the spectral emission curve given by Kampa and Boden (1957, p. 88, fig. 8). According to these authors, the emission spectrum of *P. atlanticum* is bimodal, with a primary peak at about 480 mμ, and a smaller secondary peak near 525 mμ. The limits of this curve are far above the base-line, and it has been arbitrarily assumed that light emission extends from 420 to 600 mμ. In this respect it would resemble the blue light of other pelagic animals. Under mechanical stimulation, luminescence lasts up to 17 sec. At a distance of 1 m (in air), estimated radiant flux ranges from $1\cdot4 \times 10^{-8}$ to $1\cdot4 \times 10^{-6}$ μW/cm^2 receptor surface. Kampa & Boden (1957) report light intensities of $0\cdot8 \times 10^{-2}$ to 4×10^{-2} μW/cm^2 at a distance of 1 cm (recalculated, according to the inverse square law, as $0\cdot8 \times 10^{-6}$ to 4×10^{-6} μW/cm^2 receptor surface at 1 m in air).

Pyrosomids are warm-water planktonic organisms, usually found at depths of less than 500 m, but which have been taken as deep as 2850 m. *P. atlanticum*, in its various subspecies, is cosmopolitan in distribution. *P. spinosum* is found in the Atlantic, Indian and Pacific Oceans (Ihle, 1910; Metcalf & Hopkins, 1919; Thompson, 1948; Berrill, 1950*a*).

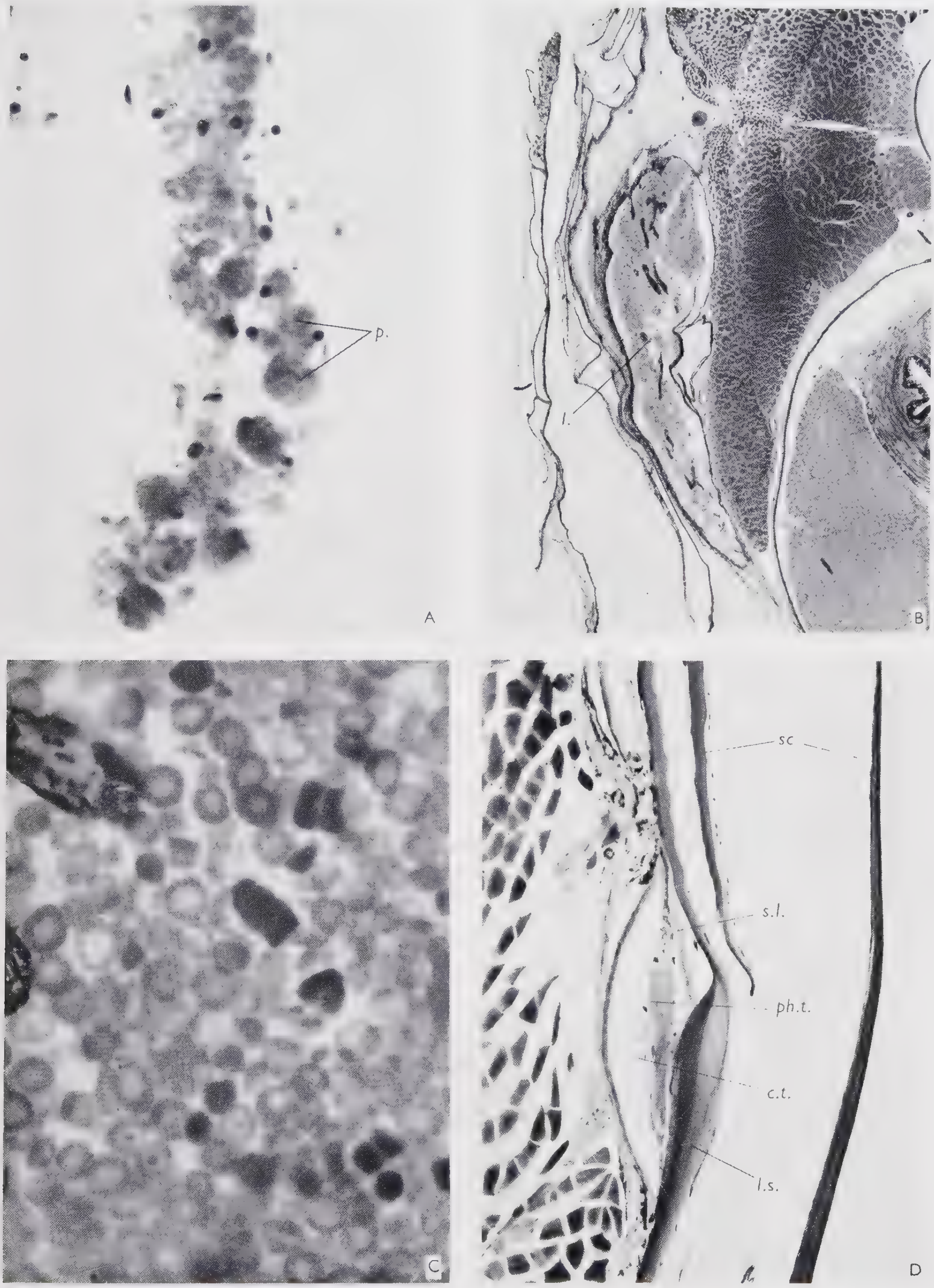
TELEOSTEI

Searsia koefoedi Parr

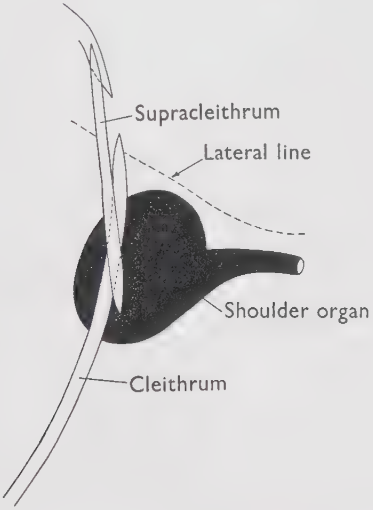
One living specimen was captured ('Sarsia' Sta. no. 23, cruise 3/57). This is a black, bathypelagic species, previously recorded from the North and South Atlantic, the Mediterranean and the Indian Ocean (Brauer, 1906; Norman, 1930; Beebe, 1933; Parr, 1937, 1951; Maul, 1948).

EXPLANATION OF PLATE I

Photomicrographs. A. Light organ of *Pyrosoma atlanticum*, showing photocytes. × 1650. B. Light gland (postclavicular organ) of *Searsia schnakenbecki*. × 25. C. Cellular contents of light gland of *S. schnakenbecki*. 'Red cells', light; 'blue cells', dark. × 1100. D. Photophore (anal organ, series *AO*) of *Myctophum punctatum*. × 265. *c.t.*, connective tissue; *l.*, light gland; *l.s.*, lenticular enlargement of scale; *p*, photocytes; *ph.t.*, photogenic tissue; *sc.*, scale; *sl.*, striated layer.

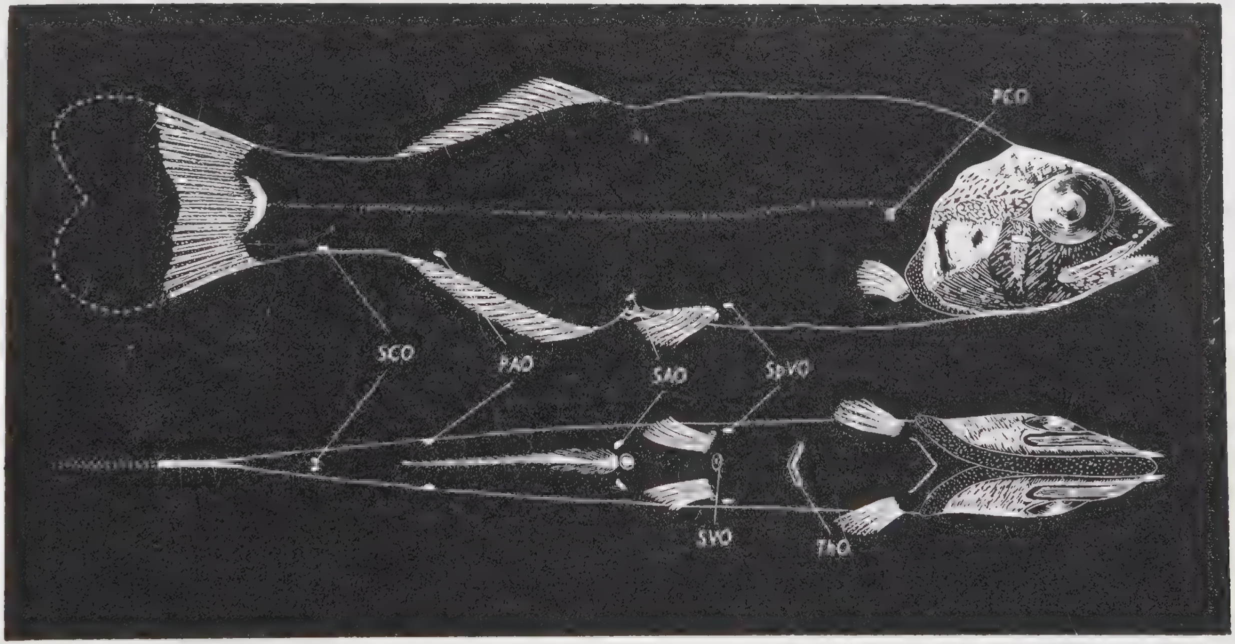

p.
A
l.
B
C
sc
s.l.
ph.t.
c.t.
l.s.
D

Light organs appear as follows (Text-fig. 10): a transverse linear thoracic organ (*ThO*), appearing as a depression with a white fleck at one end; a subventral V-shaped organ, black in hue (*SVO*); a pair of white supraventrals (*SpVO*); a pair of supra-anal organs (*SAO*); a pair of white postanals (*PAO*); and a pair of white subcaudal organs (*SCO*). The postclavicular organ (*PCO*) is obvious. Other reputed light organs, not clearly discernible in my specimen, are the submental and posterioventrals. Opercular and branchiostegal organs are faint. On the other hand, there appears to be a trace of a light organ on the basal portion of the posterior ray of the pectoral fin. Gunther (in Norman, 1930) observed that the light organs were red in life (see also Beebe, 1931; Parr, 1937). The shape of the supraclavicular process (=postcleithral or postclavicular organ) is illustrated by Beebe (1933) and shown in Text-fig. 9.



Text-fig. 9. Diagram of the postclavicular or shoulder luminescent organ of *Searsia* (=*Bathytroctes*) *rostratus*, copied from Beebe, 1933. The original drawing was made from a cleared specimen 32 mm long.

The animal was alive when brought to the surface, and it was seen to discharge a bright luminous cloud into the water when handled. The light appeared as multitudinous bright points, blue-green in colour. On repeated stimulation the light became weaker and ceased, and the fish died. Computation of radiant flux was carried out as described for *S. schnakenbecki* on p. 792). The luminous glow lasted about 4 sec. At a distance of 1 m (in air), light intensity was about 2×10^{-6} μW/cm^2 receptor surface (Tables 2 and 3). The light of this specimen of *S. koefoedi* was about 5 times brighter than that of *S. schnakenbecki*, as measured in these few instances.



Text-fig. 10. Lateral and ventral view of *Searsia koefoedi*. Legend for light organs: *PCO*, postclavicular (shoulder) organ; *ThO*, transverse thoracic organ; *SVO*, subventral organ; *SpVO*, supraventrals; *SAO*, supra-anal organs; *PAO*, post-anal organs; *SCO*, subcaudal organ. ½ natural size.

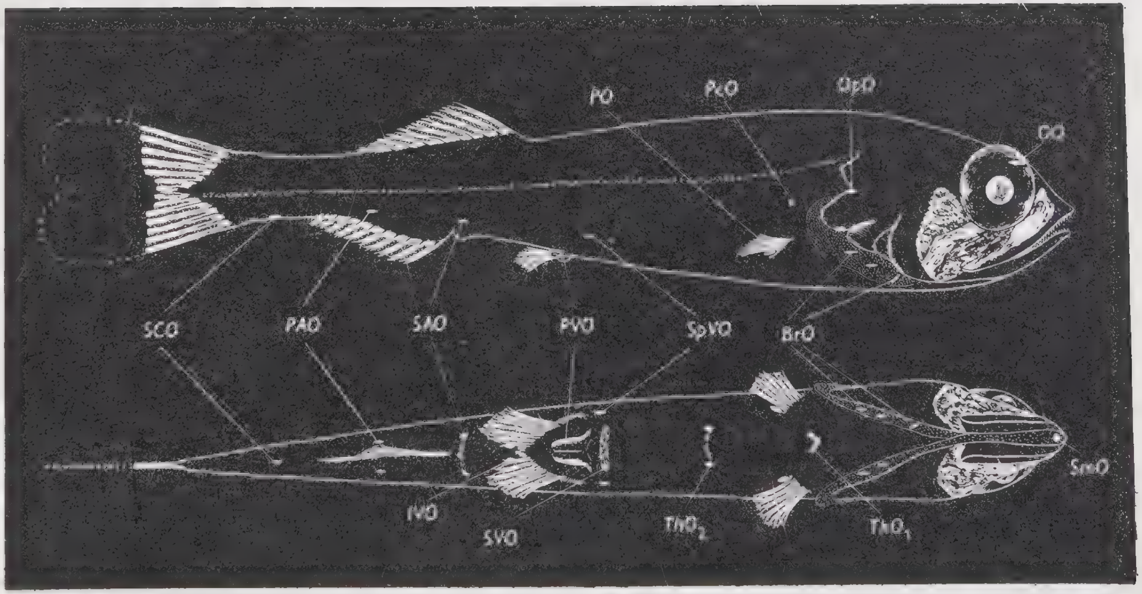
Searsia schnakenbecki Krefft

One living and several dead specimens were captured ('Sarsia' Sta. no. 5, cruise 3/57). This fish provided a spectacular luminescent display. When gently stimulated by being lightly pressed with the fingers, it suddenly shot forth myriads of blue-green sparks into the water. These brightly illuminated the whole dish with a glow lasting about 2 sec. The intensity of light was much greater than that usually recorded, and it was necessary to employ a piece of Chance neutral glass as a reducing filter when recording the responses. With repeated stimulation, the light gradually became weaker, and ceased after about six discharges. Since the fish lived only a short time, it was not possible to determine the spectral composition. In calculating light intensities, I made use of a spectral curve resembling that for *Beroë* light, but with the maximum displaced to 500 mμ. At a distance of 1 m (in air), estimated values for radiant flux are 150×10^{-9} and 430×10^{-9} μW/cm^2 (Tables 2 and 3, pp. 711 and 713. For light with maximal emission at 480 mμ, estimated radiant flux would range from 130×10^{-9} to 365×10^{-9} μW/cm^2.

Searsia schnakenbecki was described by Krefft (1953) from specimens captured in the region 63° 20′–40′ N., 11–12° W., depth of water 450–500 m. It is probably bathypelagic in habits. My specimen has the following suppositional light organs, which largely agree with comparable organs described in the type specimen (Krefft, 1953): submental (*SmO*); seven branchiostegal organs (*BrO*); eye organ (*OO*); opercular organ (*OpO*); pair of pectoral organs (*PO*); transverse thoracic organ 1 (ThO_1); transverse thoracic organ 2 (ThO_2); pair of supraventrals (*SpVO*); transverse subventral (*SVO*); a posterioventral (*PVO*); a pair of inferior ventral organs (*IVO*); a pair of supra-anal organs (*SAO*); a pair of postanals (*PAO*); and a subcaudal organ (*SCO*). In addition, there is a black shoulder organ (postclavicular organ). The position of these various organs is shown in Text-fig. 11.

Most of the surface of the fish is black, except for clear cheeks and cranial roof. The light organs *SmO*, *BrO*, *OO*, *OpO*, *PO*, *SpVO*, *IVO*, *PVO* and *SCO* appear as white spots or flecks on a black ground. Transverse thoracic organ 1 (ThO_1) is grey in colour, V-shaped, with the apex of the V pointing anteriorly. Transverse thoracic organ 2 is a grey transverse stripe with a white fleck at either end. The transverse subventral organ (*SVO*) is a broad grey transverse stripe. The posterioventral organ is a raised wedge-shaped mound, black in colour. The postcleithral organ *PcO*) is a raised papilla lying on the lateral body wall above the pectoral fin.

All these organs have been called light organs, although they have not been seen luminescing in the living fish. Parr (1951) described the shoulder or postcleithral organ of the Searsidae as a large sac lying underneath the skin, often extending forward partly underneath the upper posterior portion of the cleithrum, and opening to the exterior by a tube. The sac has a black lining,


PO
PcO
OpO
OO
SCO
PAO
SAO
PVO
SpVO
BrO
SmO
IVO
SVO
ThO_2
ThO_1

Text-fig. 11. Lateral and ventral view of *Searsia schnakenbecki*. Legend for light organs: *SmO*, submental; *BrO*, branchiostegal; *OO*, eye organ; *OpO*, opercular organ; *PO*, pectoral organ. ThO_1, transverse thoracic organ 1. ThO_2, transverse thoracic organ 2; *SpVO*, supraventrals; *SVO*, transverse subventral; *PVO*, posterioventral; *IVO*, inferior ventral; *SAO*, supra-anal organ; *PAO*, postanal organs; *SCO*, subcaudal organ; *PcO*, postclavicular (shoulder) organ. ½ natural size.

and is traversed from outer to inner wall by irregular strands or columns of soft tissue; it discharges through a tube, which also has a black lining. Parr (1951) and Tucker (1954) suggest that the function of this organ lies in the discharge of a luminous secretion, which I have observed.

Histological observations were made on the luminescent gland of *S. schnakenbecki*. The specimen of *S. schnakenbecki* was fixed in formalin initially, and some time later was post-fixed in Wittmaack's fluid. Sections were cut in celloidin at 15 μ, and treated with a trichrome stain (iron haematoxylin, Bordeaux red, and aniline blue).

The luminescent gland lies deep in the dermis, immediately outside the skeletal musculature (Pl. I B). It is elongated dorsoventrally, and is about one-fifth the height of the fish (about 2·3 mm high and 0·5 mm wide). The walls and internal trabeculae consist of a thick layer of dense connective tissue, staining with aniline blue. This tissue contains a dense population of yellowish black (by transmitted light) melanophores, some of which extend, as flattened cells, over the internal surface of the gland. There is no recognizable continuous epithelial lining.

In the connective tissue walls of the gland and in the trabeculae there are small 'violet cells' (Pl. I C). These are scattered individually, or grouped in clumps which give the impression of originating by division. Many of these cells also occur on the internal surface of the gland, and a few in the internal lumen; some of the cells seem to be in the process of migrating through the connective tissue into the lumen of the gland. The cells are coloured deep violet by the stain; the nucleus is very dense and dark, and the basophilic cytoplasm contains purple granules. These violet cells are only about 5 μ in diameter, and the cytoplasm is rather sparse.

Lying in the lumen of the gland are many spherical 'red cells'. These are large, ranging from 8 to 40 μ in diameter. The body of the cell or spherule (the 'cytoplasm') is packed with acidophilic granules, coloured pink by the dye; staining affinity is rather weak. In the centre of the spherule is a clear area, representing the nucleus, and containing a few fine chromatic threads.

A third cell type is found in the gland lumen, the 'blue cells'. These are smaller in size, around 6–8 μ, the nucleus is more prominent and deeply staining, and the basophilic cytoplasm contains blue granules. All kinds of transitional stages appear to exist between the 'blue' and 'red' cells, the nucleus in such intermediates becoming fainter, the cell volume increasing, and the cytoplasm shifting from basi- to acidophilic.

The picture that emerges is the following. The large acidophilic spherules appear to be the photogenic bodies. Presumably, when expelled, they become disrupted and activated and they then emit light. There is a reasonable degree of transition between the small 'violet' cells in the gland wall and the large 'red' cells in the gland lumen, and it is not improbable that the violet basophils represent the generative tissue. The latter cells, passing into the gland lumen, gradually become transformed into the acidophilic spherules. This picture is rather an extraordinary one, and is offered with some hesitation, since a closely comparable situation has not been described in other luminescent animals producing a luminous secretion. Perhaps a distant analogy can be drawn with the proliferating epithelial tissue within the testis, or the sebaceous glands of mammalian skin. There is no continuous epithelial lining in the light gland of this specimen, however. Although this appearance may be an artifact, due to glandular discharge, fixation, etc., this is rather unlikely. Rather the generative cells appear to originate in the connective tissue itself, and subsequently pass into the gland lumen. In two other luminous teleosts (*Malacocephalus* and *Monocentris*), luminous secretion is produced by break down (cytolysis) of epithelial cells lining the light organs (Hickling, 1926; Okada, 1926); Harvey (1952) implicates bacteria in the luminescence of these two fish.

Myctophum punctatum Rafinesque

Lantern fish were attracted to the side of the boat by hanging a lamp over the side after dark. Fish which came to the surface were captured in a hand net. All specimens were *M. punctatum.* Position 47° 00′ N., 6° 05′ W. ('Sarsia' Sta. no. 8, cruise 3/57).

The myctophids examined were 8–9 cm in length (snout to tip of tail). This species possesses many large photophores on head and trunk, and the photophores have been labelled and numbered for systematic purposes (see Fraser-Brunner, 1949, fig. 2). Myctophids flash periodically when kept in aquaria. The light appears blue to the eye. To record luminescence, a fish was placed in a small dish beneath the photomultiplier, and was stimulated mechanically by handling it. With this treatment, the fish responds by lighting up all its photophores.

The spectral composition of myctophid light is not known, and I used the spectral curve of *Chaetopterus* light (Text-fig. 4, p. 714) for purposes of calculation. Since the light of both animals is blue, and since the photomultiplier has a fairly flat response below 500 mμ, any error occasioned by this procedure will be small.

Values for calculated radiant flux are given in Tables 2 and 3 (pp. 711 and 713). The light was measured at a distance of about 95 mm, and the recordings are of the light emitted by about half the photophores borne by the fish. The light is emitted in periodic flashes, sometimes grouped into rhythmic bursts. Each flash lasts about 0·5 sec and bursts of flashes last up to 3–4 sec (Text-fig. 3, w_1, w_2). At a distance of 1 m in air, minimal estimates of radiant flux are 1×10^{-9} μJ to 50×10^{-9} μW/cm^2 receptor surface. Owing to the large size of the source, viz. a lantern fish with all its photophores glowing, decrease of intensity with distance is less than that predicted by the inverse square law; consequently, these values for radiant flux may be slightly low. There is a further difficulty owing to the circumstance that the photophores of lantern fish possess lenses or focusing devices, the optical characteristics of which are unknown; it is highly unlikely that all the photophores bring their light into focus in the same plane. For these various reasons, recalculated estimates of radiant flux at distances other than those actually measured can be regarded only as a rough estimate.

With regard to their histology, the photophores of *Myctophum* and *Scopelus* have been examined microscopically by earlier workers (Emery, 1884; Brauer, 1908; Ohshima, 1911). My own observations were made on the anal organs (series *AO*) of *Myctophum punctatum*. These were fixed in Bouin's, cut in celloidin, and stained with Ehrlich's haematoxylin and eosin, or with a trichrome stain containing iron haematoxylin, Bordeaux red, and aniline blue.

A photophore is overlapped externally by 1 or 2 scales, and the scale immediately overlying the organ is intimately associated with its structure (Pl. I D). At the surface of the light organ the scale is thickened into a lenticular swelling; there is the usual epidermal covering. Beneath the scale lies a striated layer (the 'schüllelformige Organe' of Brauer and the 'peculiar membrane' of Ohshima). In section this shows transversely arranged striae or fibrils, which stain blue with haematoxylin. Underneath the striated layer is a patch of photogenic tissue, consisting of narrow elongated cells, with long axes directed dorsoventrally. These have narrow elongated nuclei, the cytoplasm has a somewhat indistinct fine granular appearance and stains blue with haematoxylin. The photogenic cells are in the neighbourhood of 40–50 μ long; although cell boundaries are difficult to distinguish, the cells seem to be about 5 μ broad.

The photogenic mass is invested with a thin limiting membrane of flattened cells, possibly a connective tissue sheath (Pl. I D). Filling the intervening space, between the photogenic mass and the external sheath, is a loose connective tissue (mucous connective tissue of Emery, gelatinous layer of Brauer and Ohshima). This has a faintly eosinophilic ground substance, containing flattened nuclei. Investing the organ externally is a dense connective tissue sheath, consisting of an internal homogeneous eosinophilic layer, and an external dense basophilic layer. Occasional pigment cells, or extensive streaks of black pigment, are found behind the connective tissue sheath. The organ is ringed with pigment cells, which extend a short distance down over the dorsal face of the organ. The arrangement of the pigment and the asymmetrical arrangement of the lens and photogenic tissue all indicate that the light from these organs is directed downwards.

Since the lens system and aperture are wider than the actual photogenic tissue, they will have the action of enlarging the apparent source. In addition, the lens may have a collimating effect.

Leptostomias sp.

A *Leptostomias*, close to *L. macropogon* Norman, was captured alive, at 'Discovery' Sta. no 3344, on 18 October 1955. It was observed in the dark room. Notes made at the time read: 'The long chin barbel had an enlarged cream coloured tip. When the fish was sharply displaced, or the tentacle tip was gently stroked with a camel-hair brush, the tentacle tip gave off a faint bluish luminescence. Faint glow, lasting about a second.'

L. macropogon has been captured previously in the South Atlantic (Norman, 1930).

The barbel bulb is also yellow in fresh specimens of *L. gladiator* and *L. bermudensis*; the luminescence, apparently, has not been seen. The barbel of a living *Chirostomias pliopterus* gave off a steady pink glow anteriorly, and a white glow posteriorly. The bulb of a living male *Eustomias bibulosus* gave off a distinctly green light in three brilliant flashes (Beebe & Crane, 1939). Beebe (1935) observed red and blue lights in the barbels of deep-sea melanostomiatids during dives in his 'bathysphere'.

LUMINESCENCE AND REFLEXION OF LIGHT FROM ANIMAL SURFACES

It is well known that many animals from bathypelagic waters, below the level of light penetration, are black or red in colour, e.g., Scyphomedusae, nemertines, Crustacea, and teleosts. In upper, dimly lit waters, some animals are translucent, motley coloured, or have a silvery sheen. Thus silvery and pale-coloured fishes are mostly found in the upper 500 m, black and brown species at greater depths. This correlation of colour with depth is not absolute, but is striking enough when examining net hauls from various depths, and there has been much speculation concerning its significance (Murray & Hjort, 1912; Marshall, 1954). The commonest suggestion is that dark colours (red, black and brown) serve to reduce reflexion to a minimum in deep water, and thus decrease the animal's chances of being seen. With these ideas in mind it seemed worth while to make some actual measurements of reflexion from bathypelagic species.

Few attempts have been made to measure reflexion from animals, although many curves are available for reflexion characteristics of plants (leaves, flowers, etc.). Using a Hardy photoelectric recording spectrophotometer, Edwards & Duntley (1939) made a detailed study of pigments and colours of human skin. They measured reflexion from the skin surface, transmission through the skin and absorption by the skin and its contained pigments. Deanin & Steggerda (1948) have used the same instrument for measuring melanin dispersion in the frog.

Measurements of reflexion were made with a Unicam instrument, namely the 'diffuse reflectance attachment' (S.P. 540) coupled to a 'photoelectric quartz spectrophotometer' (S.P. 500). This instrument measures diffuse reflectance, using a block of magnesium carbonate as a reference standard. The reflexion coefficient of $MgCO_3$ ranges from 0·967 at 450 mμ to 0·989 at 650 mμ (*Handbook of Chemistry and Physics*, 1949). The characteristics of the instrument were essayed by measuring reflectance from green leaves: two resultant curves for peony leaf (A) and *Euonymus* leaf (B) are shown in Text-fig. 12. The reflectance characteristics of these leaves are determined in large part by the properties of their chlorophylls and carotenoids; reflectance is less in the more glossy leaf of *Euonymus*, as might be anticipated. The instrument measures very little direct reflexion. At 450 mμ, reflexion from a disc of polished silver was 6 %, compared with published values of 0·90 for reflexion factor (direct) (Walsh, 1953).

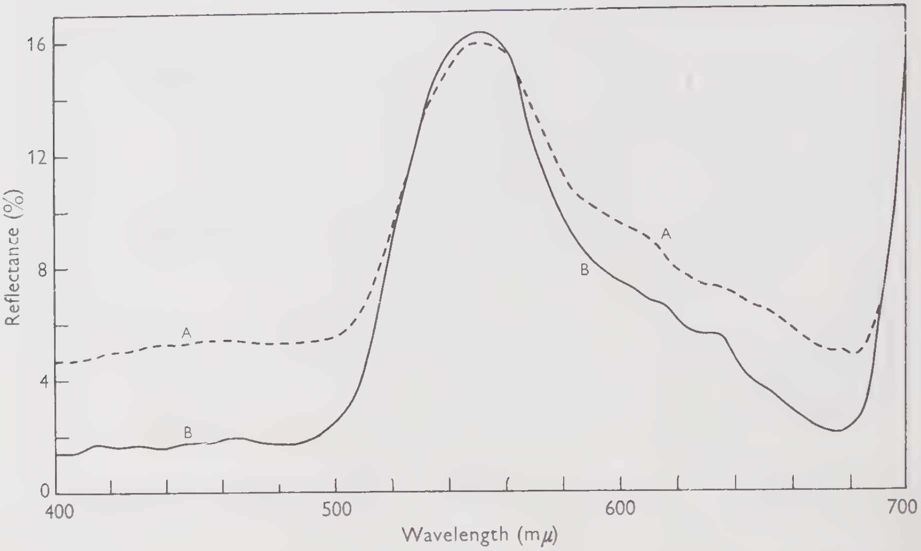
CRUSTACEA

Measurements were made of reflexion from the integument of the deep-sea shrimp *Acanthephyra multispina* ('Sarsia' Sta. nos. 5 and 10, cruise 3/57). The data are plotted in Text-fig. 13 (curves D and E). Both *A. purpurea* and *A. multispina* are deep red in colour, owing to a heavy deposition of carotenoids in the body coverings. Frozen specimens of *A. multispina* were saved for pigment extraction and treated as follows. After thawing the animals the integument was stripped off and extracted with acetone. Water and ethanol were added, and the pigment was transferred to petroleum ether (b.p. 40–60° C). Absorption was read in a spectrophotometer (Unicam S.P. 600). The solvent was evaporated *in vacuo*, and the pigment redissolved in CS_2. Absorption was read in the spectrophotometer. Absorption curves in petroleum ether and CS_2 are shown in Text-fig. 13 (A, B). These curves are identical with those for astaxanthin in the literature (Karrer & Jucker, 1948; Goodwin, 1952).

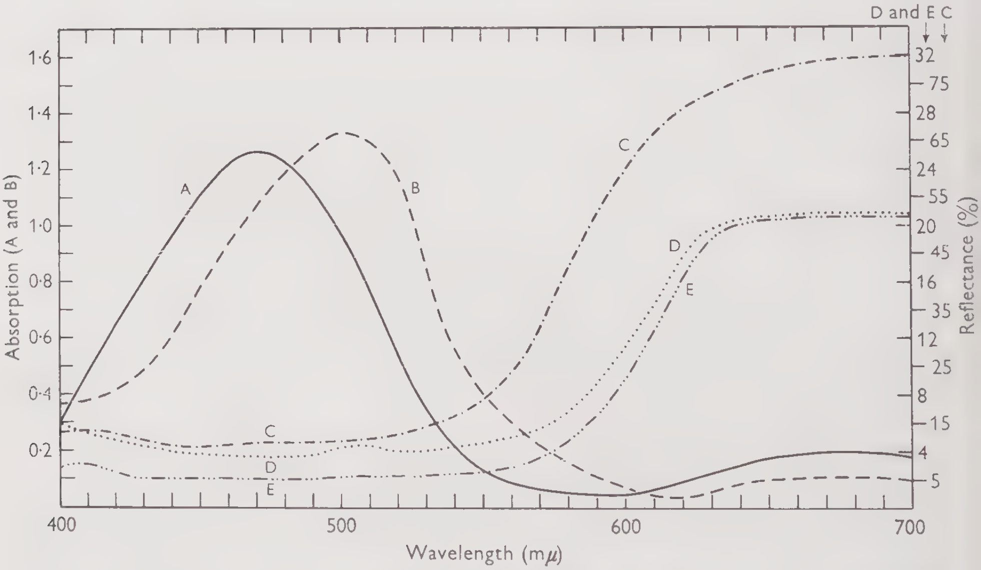
A piece of white filter-paper was soaked in the pigment solution and dried. Reflectance of the coloured paper was read in the spectrophotometer (Unicam S.P. 540 + S.P. 500). A curve for diffuse reflectance (corrected for reflexion characteristics of the paper itself) is shown in Text-fig. 13 (C).

Carotenoids sometimes occur as carotiprotein complexes, for example crustacyanin, the blue carotenoid-protein of lobster shell. Wald, Nathanson, Jencks & Tarr (1948) have succeeded in extracting the latter by employing dilute citric acid. I tried grinding up the integument of *A. multispina*, and extracting with 0·1 M citric acid. No pigment was removed by this process, indicating that the astaxanthin occurs in a free state.

A. purpurea and *A. multispina* are deep red in colour except for the black eyes. The entire surface of the body is covered with greatly expanded chromatophores having long filamentous processes. The cuticle itself is pigmented, appearing amber yellow between the chromatophores. In *Systellaspis debilis*,



Text-fig. 12. Reflectance curves for green leaves of peony (A), and Japanese *Euonymus* (B), as determined with the Unicam reflectance attachment (S.P 540).



Text-fig. 13. Spectral curves relating to *Acanthephyra multispina*. A and B. Absorption curves for carotenoid pigment (astaxanthin) extracted from the integument: A, in petroleum ether; B, in CS_2. 1 cm cells. Figures at the left for curve A (readings in petroleum ether), × 10; for curve B (readings in CS_2), × 5. C. Reflectance of filter-paper impregnated with astaxanthin. D, E. Reflectance from surface of the animal. Scales for C, D and E shown at the right.

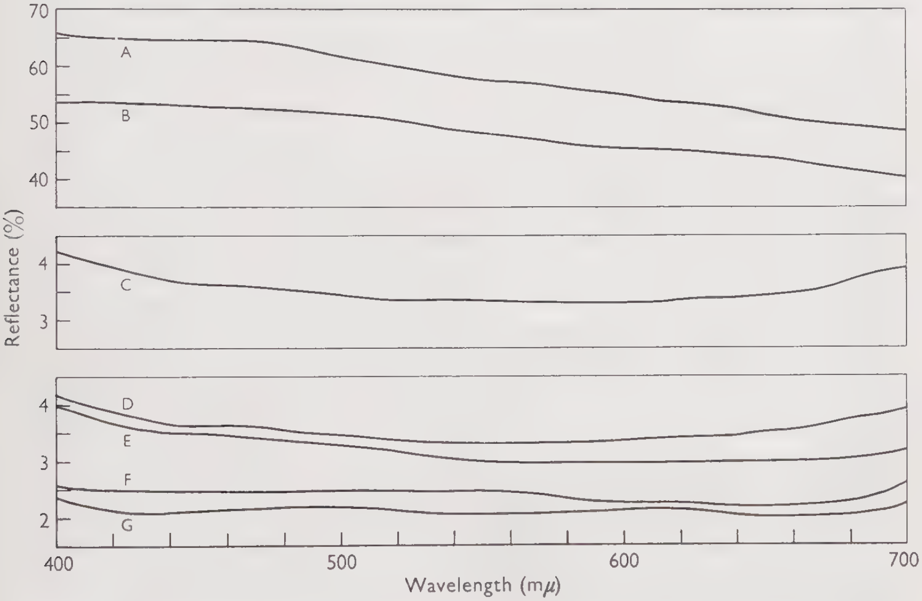
Dennell (1955) found that the deep red pigment of the body was confined to the cuticle.

The characteristics of the reflectance curves (Text-fig. 13) show good agreement with the absorption properties of extracted pigment, which seems to be astaxanthin. The reddish coloration of *Acanthephyra* is thus produced largely by the red astaxanthin pigment, located in red chromatophores and occurring freely in the cuticle.

TELEOSTS

The reflectance of several species of deep-sea fish was measured, and the data are assembled in the curves of Text-fig. 14. Specimens were: *Argyropelecus olfersi* Cuv. ('Sarsia' Sta. no. 5, cruise 3/57); *Cyclothone* sp. ('Sarsia' Sta. no. 6, cruise 3/57); *Xenodermichthys copei* Gill ('Sarsia' Sta. no. 10, cruise 3/57); and *Chauliodus* sp. ('Sarsia' Sta. no. 5, cruise 3/57).

The *Cyclothone* was a black bathypelagic species. Two sets of measurements showed low reflectance, ranging from 3 to 4% in the visible range (400–700 mμ). Reflectance is maximal in the blue, falls off slightly at longer wavelengths in the green and yellow, and shows a slight rise again in the red (above 650 mμ).



Text-fig. 14. Reflexion from the integument of some deep-sea fish. Measurements made with Unicam instrument (S.P. 540 + S.P. 500). Ordinates, reflexion as percentage of $MgCO_3$ standard (maximum = 100). Abscissae, wavelength. A, B. *Argyropelecus olfersi* Cuv, abdominal wall and trunk. C. *Xenodermichthys copei* Gill, lateral abdominal wall. D, E. *Cyclothone* sp., flank of two specimens. F, G. *Chauliodus* sp., lateral peduncle and ventral surface of trunk, respectively. Note changes of scale on ordinates.

The black pigment in the skin of *Cyclothone* is contained in melanophores, which are densely distributed over the surface of the body. In freshly caught specimens these are all expanded, and their processes touch one another, thus forming a mosaic. Several shapes of melanophores can be recognized: cells with fine irregular, branching and radiating processes; cells with a dark pigmented centre from which radiate lobate processes; and more or less hexagonal-shaped pigmented cells, in which the processes are short or wanting. The melanophores lie in two layers, the more superficial cells having fine branching processes, while the deeper cells have coarser lobate processes, or are hexagonal in shape. The fin rays possess a layer of black pigmented cells on either side, and the ventral photophores lie in black pigmented capsules. There is no trace of other kinds of chromatophores, neither guanophores nor lipophores. Light shines through and between the processes of the cells, the latter being only partially absorbing. The muscles are translucent; the visceral region, owing to a black peritoneum, and much of the head, are opaque.

Pieces of fins of freshly caught (dead) specimens were placed in fish-Ringer, to which was added adrenaline to make 1/100,000 and 1/10,000. No contraction of the melanophores occurred in 1 h. Adrenaline is a strong stimulant of chromatophores of coastal and freshwater fish, causing concentration of the pigment. It is possible that the chromatophores of deep-sea fish, permanently expanded in a dark environment, no longer possess a mechanism for becoming concentrated.

Black melanin pigment was extracted from the following species, and absorption characteristics determined: *Cyclothone livida* Brauer ('Discovery' Sta. nos. 3366, 3372, 3375); *Gonostoma denudatum* Rafinesque ('Discovery' Sta. no. 3372).

Specimens were kept frozen until they could be used. They were then treated with ether:ethanol (1:1) for 24 h, washed with ethanol, followed by water, and digested in boiling 0·05 N NaOH under a reflux condenser for 3 h. Solutions were straw-coloured to reddish brown. They were filtered and the pigment precipitated by addition of strong HCl. Upon centrifugation the melanin pigment was thrown down as a reddish brown deposit. The pigment was washed with distilled water and dissolved in ethylene chlorhydrin. When the solution was passed through a column of magnesia, the pigment was left as a brownish band at the top. The column was washed with water, extruded, and the coloured region cut off. The latter was dissolved with strong HCl, in which the pigment precipitated. The precipitate was centrifuged, and the centrifugate washed with water. Pigment-aliquots dissolved in ethylene chlorhydrin and boric-borate buffer at pH 9; these were examined in a spectrophotometer (Unicam S.P. 500). Specimens of *Cyclothone*, preserved in formalin for 1 month, were extracted in a like manner (Gortner, 1911; Lea, 1945; Serra, 1946; Smyth, Porter & Bohren, 1951).

Representative absorption curves for the pigments are shown in Text-fig. 15.

They are typical of melanin curves: melanin is a reddish pigment showing high absorption in the blue, and minimal absorption at long wave-lengths. Visceral (peritoneal) and skin pigments are similar (Text-fig. 16); formol causes little or no change in the absorption characteristics of the melanin-pigment (Text-fig. 16).

When Text-figs. 14 and 15 are compared, it will be apparent that the melanin of the skin contributes very little to the spectral composition of the reflected light; the slight rise in the reflectance curves, about 600 mμ, amounting to about 1 %, is caused by melanin in the skin. The surfaces of black bathypelagic species measured showed very low reflexion: *Chauliodus*, 2–2½ %; *Cyclothone*, 3–4 %; *Xenodermichthys*, 3–4 %. Much of this reflexion probably originates in surface tissue lying between the chromatophores. Indirect reflexion is much higher in *Argyropelecus*, which possesses a silvery surface, around 40–65 %.

DISCUSSION

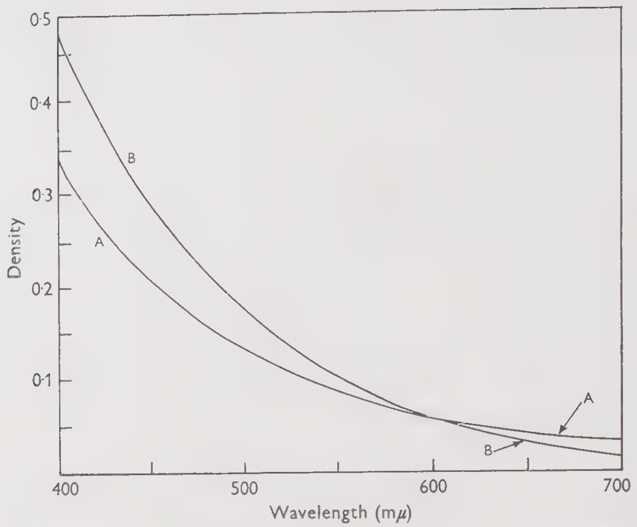
Recent estimates of the intensity of light emitted by marine animals are collated in Table 3 (p. 713). For comparison, they are brought to a common basis of energy units/cm^2 receptor surface at a distance of 1 m. Although this procedure involves some unknown degree of error, owing to probable deviation from the inverse square law, it at least provides rough estimates of orders of magnitude.

The estimates in Table 3 are all derived from photoelectric measurements. There are, in the literature, some earlier estimates of the brightness of animal light, based on visual comparison.

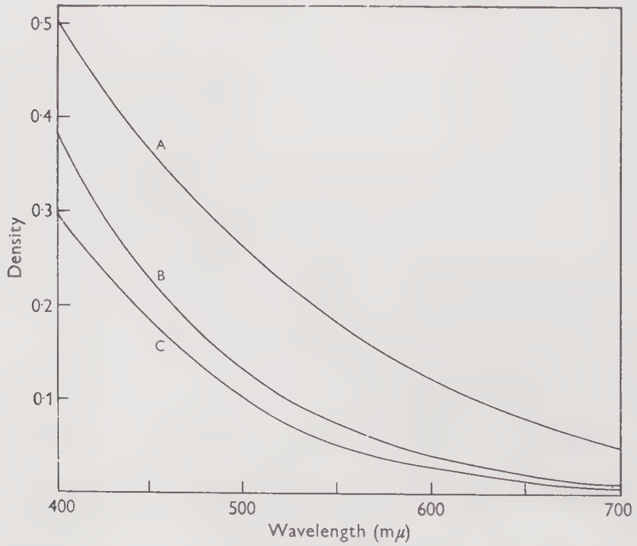
Dennell (1955) cites a figure of 0·01 mlam for the luminance of bathypelagic decapod Crustacea, such as *Systellaspis* and *Hoplophorus*. On the assumption that the visible light-emitting surface is about 10 mm^2, this value for luminance is approximately equivalent to $3{\cdot}18 \times 10^{-4}$ m lumen cm^2 receptor surface and $2{\cdot}3 \times 10^{-3}$ μW/cm^2 receptor surface at a distance of 1 cm (Dennell, 1940). This intensity is about the same as that of *Euphausia pacifica* (see Kampa & Boden, 1957) and seems to be of the right order of magnitude.

According to Nichols (1924), the light of the ctenophore *Mnemiopsis leidyi* has a brightness of 0·11–0·30 mlam. For mature animals about 100 mm long, these values are roughly equivalent to radiant fluxes of 0·4 to 1 μW/cm^2 at 1 cm (Mayer, 1912; Coonfield, 1936). They are of about the same order of magnitude as recorded by Clarke & Backus (1956) (see Table 3).

The light organ of the coastal fish *Photoblepharon palpebratus*, according to Steche (1907), has a brightness equivalent to a surface illuminated with 0·0024 Meter-Kerze, or 0·0022 lx. The light organ has a flattened oval shape, 11 × 5 mm in dimensions; the light is produced by symbiotic luminescent bacteria. The light is reported to be greenish blue in colour, and probably is not greatly different in relative spectral composition from the curve for the



Text-fig. 15. Absorption characteristics of the melanin pigment from the integument of *Gonostoma denudatum*. 1 cm cuvette in Unicam spectrophotometer, S.P. 500. A, in boric-borate buffer, pH 9; B, in ethylene chlorhydrin.



Text-fig. 16. Absorption characteristics of the melanin pigment from the deep-sea fish *Cyclothone livida*. A, from entire animal, in ethylene chlorhydrin; B, formol-preserved integument, in ethylene chlorhydrin; C, viscera, in ethylene chlorhydrin. 1 cm cuvette in Unicam spectrophotometer, S.P. 500.

relative luminous efficiency of radiation (human scotopic vision). An estimation of equivalent radiant flux is about 3×10^{-4} μW/cm^2 receptor surface at a distance of 1 cm.

The light of most pelagic animals is blue in colour, although there are notable exceptions. Actual measurements of spectral composition give maximal emission peaks (approximately) as follows: *Gonyaulax polyedra*, 478 mμ (Hastings & Sweeney, 1957); *Atolla wyvillei*, 470 mμ; *Vogtia glabra*, 470 mμ; *Beroë ovata*, 510 mμ; *Euphausia pacifica*, 476 mμ (Kampa & Boden, 1957); *Pyrosoma atlantica*, 482 mμ (Kampa & Boden, 1957). Of these animals only the light of *Beroë ovata* is green. Symbiotic bacteria from the deep-sea fish *Coelorhynchus* (*kishinouyei*?) emit light with a maximum $\nu = 21{,}200$ cm^{-1} or $\lambda = 472$ mμ (Takase, in Harvey, 1952). The bacterial light of *Malacocephalus laevis*, another macrourid, is said to have an emission maximum around 510 mμ (Haneda, in Harvey, 1952). Harvey (1952) cites some instances of lights of other colours in pelagic animals: *Thaumatolampas diadema* is a squid having photophores emitting red light; *Echiostoma tanneri* is a teleost possessing cheek organs which shine with a pink *or* blue glow (cf. Beebe & Crane, 1939). The smaller photophores of *Echiostoma* and those of *Argyropelecus olfersi* are said to emit a yellowish or yellow-green light (see Harvey, 1952).

Attenuation of light from a point source in sea water depends both upon distance (operation of the inverse square law) and absorption (given by the extinction coefficient). Clarke & James (1939) give the following values for absorption in (preserved) Sargasso Sea water:

λ (mμ)	Transmission/m (%)	Remarks
405	97·2	Filtered
436	99	Filtered
490	97·2	Filtered
—	90	Shaken
505	95·5	Filtered
546	95·8	Filtered
550	87	Shaken

A measurement made at sea of surface water (Sargasso Sea) gave a value of 96·1% at 550 mμ.

Jerlov (1951), from measurements at sea, found that transmission/m depth for blue daylight in the Atlantic Ocean varied from a minimum of 91·5% (surface) to a maximum of 98% (at 25–50 m) (various stations). Selected values for transmission at various stations (0–50 m) are

λ (mμ)	Mean transmission/m depth (%)
400	93·3–96·8
475	95·6–96·8
500	95·1–96·8
525	93·4–94·9
550	91·3–93·7

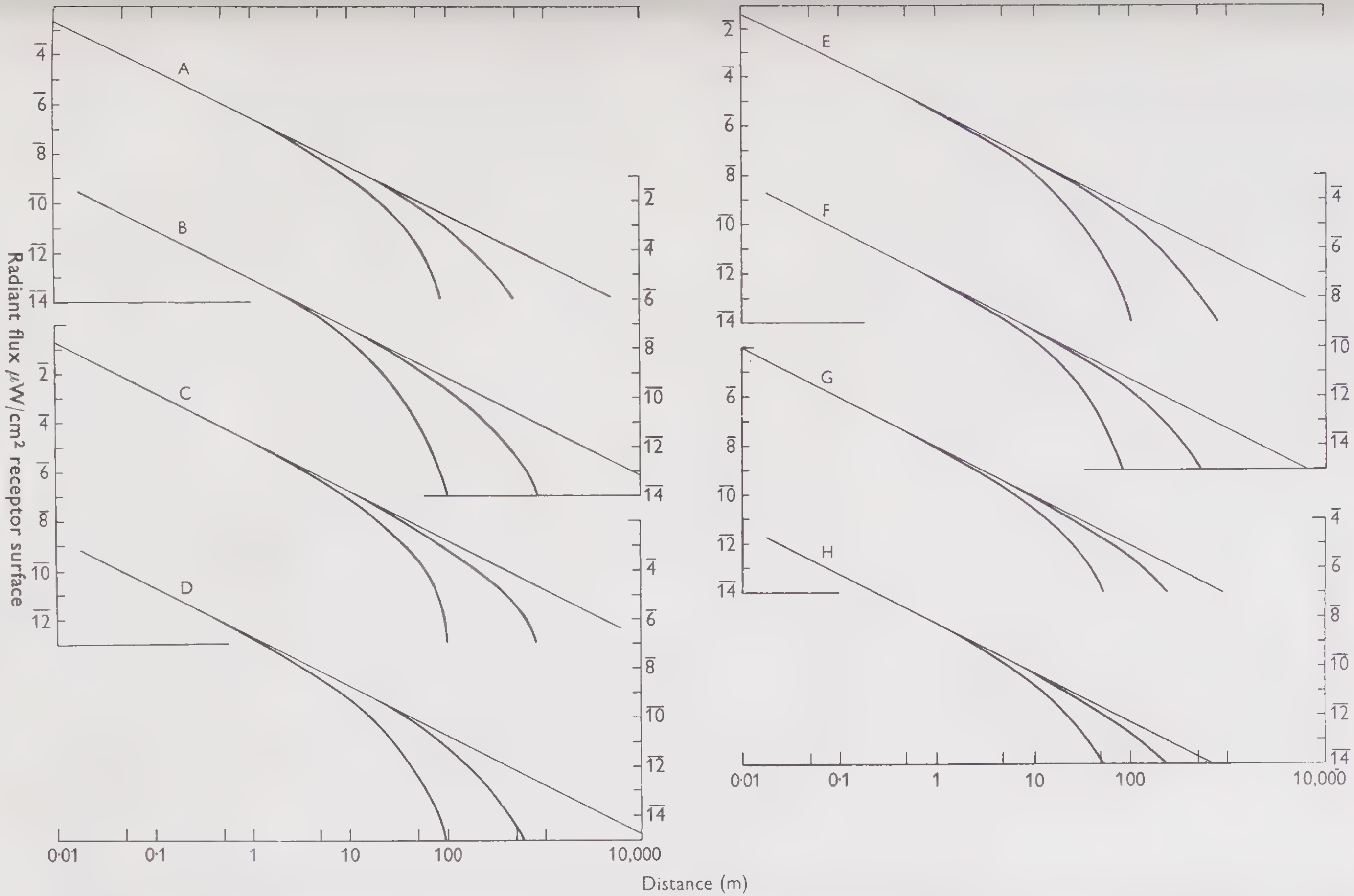
It would seem, then, that transmission in oceanic waters for blue and green light (spectral range of about 460–510 mμ) lies between 91 and 99% (transmission/m depth), and for purposes of illustration, arbitrary limits of 1 and 10% absorption/m depth have been selected, as representing two extremes for oceanic water.

Estimates of light intensities at various distances from a point source are illustrated in Text-fig. 17. The light intensities illustrated are selected from Table 3, and represent the maximal measured output of several luminescent animals in terms of radiant flux/cm^2 receptor surface at an initial distance of 1 cm, viz. of radiolarians *Cytocladus* and *Aulosphaera*; a siphonophore *Vogtia spinosa*; a scyphomedusan *Atolla wyvillei*; ctenophores *Beroë ovata* and *Mnemiopsis leidyi*; crustaceans *Euphausia pacifica* and *Acanthephyra purpurea*; *Pyrosoma*; and the lantern-fish *Myctophum punctatum*. The three curves in each figure show decrease of light intensity with distance according to the inverse square law alone, and decrease according to the inverse square law in sea water having transmission values of 99 and 90%/m depth. For these values it will be observed that the curve for 90% transmission only begins to diverge markedly from that for the inverse square law at distances greater than 10 m, and that for 99% transmission, above 100 m.

It is unfortunate that so little information exists about visual threshold or minimal intensities that can be perceived by marine animals. Earlier estimates of visual threshold for fish have made use of Grundfest's observations (1932) on *Lepomis*, a freshwater fish. These indicated that the lower limits of light intensity for fish vision were similar to those for the human eye, viz. 1×10^{-6} mlam. For an estimated pupillary area of 0·1 cm^2, equivalent radiant flux would be about $1{\cdot}2 \times 10^{-8}$ μW falling into one eye. *Lepomis* is most sensitive to the green region of the spectrum, around 530–550 mμ (Grundfest, 1932; Clarke 1936; Strickland, 1957).

The threshold of the human eye, when dark-adapted, for a small steady light source, has been estimated at 200 quanta/sec at 510 mμ (selected from values ranging from 170 to 830 quanta/sec) (Pirenne, 1956). For a just detectable flash of duration $\geqslant 1$ sec, the human eye needs 200 quanta $= 8 \times 10^{-10}$ erg to fall in the pupil, area 0·5 cm^2 in 1 sec. Pirenne (1956) suggests that the high retinal sensitivity of man is similar to that of other rod-bearing vertebrates, and that some invertebrates may also be equally sensitive. *Dendrocoelum lacteum*, a fresh-water planarian, responds to light having an energy flux of 15×10^{-9} erg/sec falling in one eye (Pirenne & Marriott, 1955).

The brightest luminescent animals known in the sea are ctenophores, Pyrosomas, and the deep-sea fish *Searsia*, all of which have a measured light output in excess of 2×10^{-7} μW/cm^2 receptor surface at a distance of 1 m. In the first two, luminescence is intracellular; the latter discharges a bright luminous secretion.



Text-fig. 17. Curves for estimated decrease in intensity of animal light at increasing distances from source. Intensities taken from Table 3. The three curves in each figure refer, from above downwards, to decrease from a point source according to the inverse square law alone; decrease in sea water having an absorption of 1 %/m; and decrease in sea water having an absorption of 10 %/m. A, *Vogtia spinosa*; B, *Beroë ovata*; C, *Mnemiopsis leidyi*; D, *Atolla wyvillei* and *Euphausia pacifica*; E, *Pyrosoma atlanticum*; F, *Myctophum punctatum*; G, *Acanthephyra purpurea*; H, *Cytocladus* and *Aulosphaera*. Scale for A on left, for B on right, and so on.

It has been suggested that the light of certain animals is used to distract the attention of other animals while its producer takes evasive action. This is probably true of various animals discharging a luminous secretion, notably pelagic decapod Crustacea, bathypelagic squid such as *Heteroteuthis*, and the bathypelagic fish *Searsia*. Presumably this behaviour pattern takes place at close quarters; when the light is bright it may be more effective in maintaining attention; its effect on distant animals, then, would be of no significance.

If we assume that marine animals, such as fish, have a visual threshold about the same as man, it is possible to make some estimates of the distance at which luminescence can be seen in clear oceanic water. Let the lowest value for radiant flux which can just be seen be $1\cdot6 \times 10^{-10}$ μW/cm^2, and let values for light intensity be the maxima given in Table 3. Then, distances at which the light of luminescent animals can be seen, in sea waters having absorption of 1 and 10 %/m may be determined from the curves of Fig. 17. These distances are listed in Table 4. A further assumption is made that the bathypelagic animals concerned have maximal sensitivity in the blue (around 470 mμ), the region of maximal emission for the luminescence of most deep-sea animals. For surface animals it is assumed that maximal spectral sensitivity occurs in the green (around 510 mμ), corresponding to the region of maximal emission of green light of animals such as ctenophores. In very clear oceanic water (absorption 1 %/m), the light of the brightest animals, viz. ctenophores and Pyrosomas, can be seen at distances from 100 to 170 m. The weaker light of coelenterates (jellyfish, siphonophores) and of euphausiids can be seen at 32–36 m. The light of *Myctophum* can be seen at 16 m.

TABLE 4. MAXIMAL DISTANCE AT WHICH THE LIGHT OF VARIOUS MARINE ANIMALS CAN BE SEEN IN SEA WATER BY AN ANIMAL WHICH CAN JUST SEE LIGHT OF $1\cdot6 \times 10^{-10}$ μW/CM^2 ENTERING ITS EYE

Species	Radiant flux in μW/cm^2 at 1 cm	Maximal distance (m) at which light can be seen in sea water having transmission of 99 %/m	90 %/m
Cytocladus and *Aulosphaera*	$5\cdot3 \times 10^{-5}$	6	4
Atolla wyvillei	2×10^{-3}	32	16
Vogtia spinosa	$3\cdot2 \times 10^{-3}$	36	20
Beroë ovata	$8\cdot5 \times 10^{-2}$	120	41
Mnemiopsis leidyi	$1\cdot9 \times 10^{-1}$	170	50
Euphausia pacifica	2×10^{-3}	32	16
Acanthephyra purpurea	8×10^{-5}	7	5
Pyrosoma atlanticum	4×10^{-2}	100	36
Myctophum punctatum	5×10^{-4}	16	10

It seems likely that animals with highly developed photophores, such as euphausiids, decapod Crustacea, and fish, make use of them as recognition signals. If the animals tend to shoal together, then the values listed in Table 4 give some idea of the maximal distances at which their lights are effective;

they can also be correlated with estimates of population density. Dennell (1955) has made an estimate of the distance at which the light from the photophores of a deep-sea shrimp can be seen by another shrimp. He gives a minimal value of 0·01 mlam for brightness, and he quotes values of 1×10^{-6} to 1×10^{-7} mlam for visual threshold of a crustacean (*Cambarus*?). Using an extinction coefficient of $u_v = 0{\cdot}0920$ for clear oceanic water, he has estimated that the light of decapod photophores may well be mutually perceived at distances of 100 m. Assume that the eye of a shrimp has an area of 0·05 cm^2; then the minimal light entering that eye which can just be seen has an intensity around 1×10^{-8} μW. One shrimp can be treated as a point source, the light from which decreases with distance according to the inverse square law. For waters having transmissions of 99 and 90%/m, the estimated maximal distance at which one shrimp could see the light of another shrimp is about 1 m. If the visual threshold of the shrimp lies at about the same level as that of man (dark adapted), the equivalent maximal distance would lie around 16–32 m.

Estimates have been made of the average spatial distribution of deep-sea shrimp, based on towing data. It is suggested that, if the animals are evenly distributed, *Systellaspis debilis* occurs at intervals of about 14 m and *Hoplophorus grimaldii* at 40 m. For a visual threshold value of 2×10^{-7} μW/cm^2, these distances are excessive for mutual perception of photophore luminescence. It is known that euphausiids occur in shoals, and, from the estimates given above, it can be argued that their lights can be mutually seen at distances of about 1 m. Since these animals appear to flash spontaneously in their natural habitat, it is likely that their lights tend to promote shoaling. There are a great many variables in these estimates. Thus, the photophores possess lenses, the optical properties of which may greatly alter the geometry of the light beam, and firmer values are desirable for visual threshold.

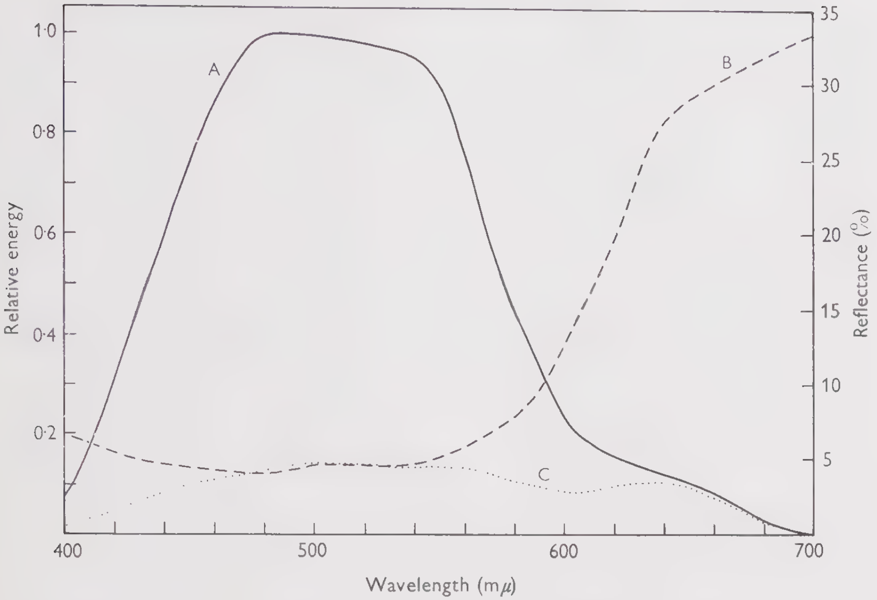
There remains for speculation a consideration of the optics of photogenic organs. Many photogenic organs obviously lack focusing devices, and the light which they emit radiates outwards more or less uniformly over some solid angle determined by the morphological configuration of the light organs. Various examples are the unicellular radiolarians and dinoflagellates; luminescent tissues of coelenterates and ctenophores; light organs of Pyrosomas; organs of Pesta (luminous liver tubules) of *Sergestes*; disc-like organs of squid *Abralia* and *Leachia*; luminous tentacles and bulbs of bathypelagic teleosts. From organs of this kind it is reasonable to assume that the light will conform to the inverse square law. In sea water, intensity will decrease in a predictable manner according to distance and transparency of the water.

Many other animals possess very complex light organs, containing reflectors and lens systems. Very little is known about the optics of these structures. If the action of the lenses is to produce a parallel beam of light, then intensity would fall off much less rapidly with distance. Trojan (1907) has ventured some conclusions concerning the optic properties of the photophore of the euphausiid *Nyctiphanes couchi*. He has found that the light is brought to a focal point immediately in front of the light organ; the light, therefore, could radiate forth from that focal point as a point source. In order to get further information about this effect, it would be desirable to make simultaneous measurements at varous distances of the light from organized photophores, containing lenses, if a suitable preparation or preparations could be found.

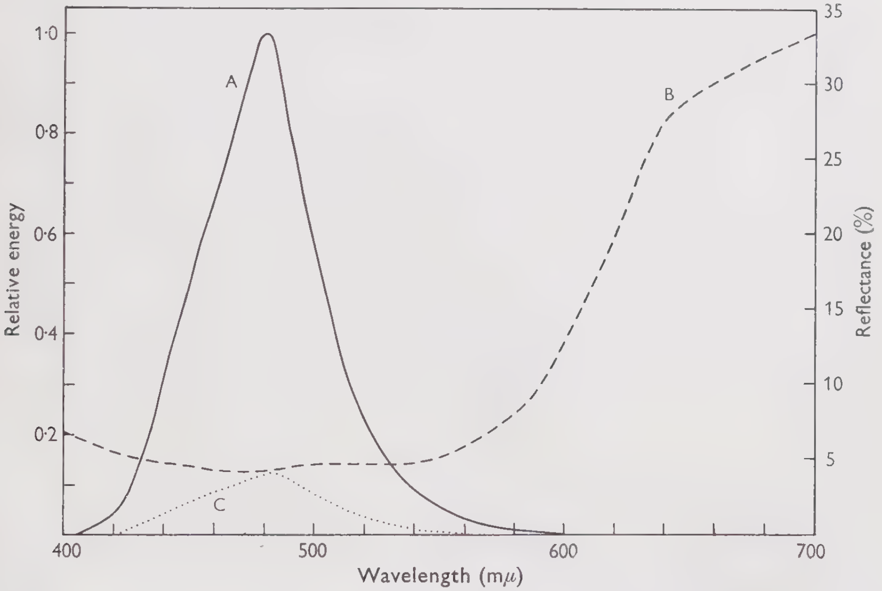
Data have been presented for reflectance of light from some deep-sea animals (Text-figs. 13 and 14, pp. 736 and 737). Reflexion curves for deep-sea fish *Cyclothone* and *Chauliodus* are fairly flat in the visual range (400–700 mμ), so that there is little change in relative reflexion at different wave-lengths (Text-fig. 14). Quite otherwise is the reflexion curve for the deep-sea shrimp *Acanthephyra* (Text-fig. 13). Reflexion from the integument of this animal is minimal in the blue, below 500 mμ, and maximal in the red, above 650 mμ. Oceanic waters are most transparent to blue light, with a maximum around 475 mμ and, owing to differential absorption, this is the only effective light in deeper regions (Text-fig. 18).

The relative spectral energy of light reflected from a surface depends on the reflectance characteristics of that surface, and the relative spectral composition of the incident light. In Text-figs. 18 and 19 are presented curves for relative spectral composition of light in clear oceanic water (from Sverdrup, Johnson & Fleming, 1942), relative spectral composition of *Cypridina* light (from Coblentz & Hughes, 1926), diffuse spectral reflectance of *Acanthephyra multispina*, and product curves (C) of incident light and reflectance. It will be seen that the integument of *Acanthephyra* reflects least light in the blue region of the spectrum, and it is this region that contains most of the energy of daylight and luminescent light in the sea. The colour of *Acanthephyra* thus seems well suited to reducing reflexion, and therefore the animal's chance of being seen.

The following estimates for *Acanthephyra* are presented to illustrate some of the factors involved in computing light reflexion from the surface of animals. Assume a luminescent fish has a light output of 1×10^{-8} μW/cm^2 receptor surface at 1 m distance, and let this light be blue, with maximal emission at 480 mμ. An *Acanthephyra*, 9 cm long, has a surface area, on one side, of approximately 12 cm^2; at 480 mμ let its reflexion be 10%. At 1 m distance, that *Acanthephyra* will receive 1×10^{-8} μW/cm^2 body surface, and reflect back $1{\cdot}2 \times 10^{-8}$ μW. At a distance of 1 m, estimated radiant flux reflected from the surface of *Acanthephyra* will be only about $2{\cdot}5 \times 10^{-13}$ μW/cm^2 receptor surface, well below visual sensitivity of known photoreceptors. In terms of the above energy values, the *Acanthephyra* would have to be within about 20–30 cm of the fish to be seen by the latter. Since the dark-coloured bathypelagic fishes also have very low reflectance throughout the visible spectrum (Text-fig. 14), similar considerations would apply to these animals.



Text-fig. 18. Curves: A, relative spectral composition of daylight transmitted through clear oceanic water, scale for ordinates on left (from Sverdrup, Johnson & Fleming, 1942); B, spectral reflexion curve for *Acanthephyra*; C, product curve of A and B. Scale for B and C on right.



Text-fig. 19. A, relative spectral composition of *Cypridina* light (from Coblentz & Hughes, 1926). B, spectral reflexion curve for *Acanthephyra*; C, product curve of A and B. Scale for A on left; for B and C on right.

Luminescent light of deep-sea animals is generally of low intensity, and reflexion from the surface of black and red species is low. It is unlikely, therefore, that luminescent species use light from barbels, tentacles, bull's eye lanterns, etc., for detecting prey, except at very close quarters. The luminescent barbels and tentacles of fish are more usually regarded as luminous fishing lures. Organs of this kind, having a light output of, say, 1×10^{-4} μW/cm^2 receptor surface at 1 cm, could be seen by other animals, possessing sensitive photoreceptors, at distances up to 10 m.

I am grateful to the Director of the National Institute of Oceanography for affording me the opportunity of going on three cruises of R.R.S. 'Discovery II' and I would thank him and his staff for many kindnesses and much assistance. Specimens were kindly identified by Dr T. J. Hart (Radiolaria), Dr F. S. Russell (medusae), Captain A. K. Totton (Siphonophora), and Mr N. B. Marshall (teleosts). Mr E. I. Butler gave valuable technical assistance. Dr D. Davenport kindly collected the Pacific specimen of *Pyrosoma atlanticum*. Mr G. N. Harding of the U.K. Atomic Energy Authority supplied the ^{60}Co-stilbene phosphor, for which I am grateful. I would thank Dr E. J. Denton and Mr F. A. J. Armstrong for advice concerning photometric computations. Part of the apparatus used in this research was purchased with grants from the Royal Society.

SUMMARY

Miscellaneous observations have been made on the luminescence of pelagic deep-sea animals. Approximate spectral emission curves have maxima as follows: siphonophore, *Vogtia glabra*, 470 mμ; scyphomedusa, *Atolla wyvillei*, 470 mμ; ctenophore, *Beroë ovata* 510 mμ. The intensity of luminescence of various species was measured. Animals with very bright luminescence are siphonophores, scyphomedusans, ctenophores, Pyrosomas, and a fish *Searsia*, which discharges a luminous secretion (fluxes up to 1×10^{-1} μW/cm^2 receptor surface at 1 cm distance). Histological descriptions of the light organs or glands of *Atolla wyvillei*, *Pyrosoma*, *Myctophum punctatum* and *Searsia schnakenbecki* are presented. Measurements of the spectral reflexion characteristics of the surfaces of deep-sea red shrimp and black fish show that these animals reflect very little blue light, such as that of luminescent light. Estimates are presented of the distances at which animal luminescence could be perceived in sea water by animals having photoreceptors as sensitive as the human eye.

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THE BUOYANCY OF BATHYPELAGIC FISHES WITHOUT A GAS-FILLED SWIMBLADDER

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(Plates I and II, and Text-figs. 1–3)

INTRODUCTION

The upper reaches of the deep ocean contain many bathypelagic fishes with a capacious, gas-filled swimbladder. But living within and below this region are also numerous species in which this hydrostatic organ is absent or markedly regressed (Marshall, in preparation). In the neritic province nearly all the fishes that swim freely at the various water levels (and can stay poised at a particular level without undue effort) have a well-developed swimbladder, the capacity of which is about equal to 5% of the body volume (Jones & Marshall, 1953). Having this amount of gas, these fishes are able to keep their weight in water close to the vanishing point. If such a fish were deprived of its swimbladder, it could keep at a constant level only by exerting a downward force equivalent to 5% of its weight in air. The swimbladder thus saves the fish the energy needed for such effort, which is quite appreciable.

The advantage to a fish of being in neutral buoyancy can be illustrated by the following simple calculations. A fish deprived of the gas in its swimbladder would have a reduced weight in water of 5–8% of its weight in air (depending on whether it were a a marine or freshwater fish). To stay at constant level it would have to exert a downward force equal to its reduced weight (and unlike terrestrial animals, pelagic creatures only exert forces on the surrounding medium by making movements). When it is remembered that even an active pelagic fish seldom exerts a force of more than 25–50% of its weight in air for more than a very brief period (Gray, 1953), the force necessary for a fish to maintain its level appears to be considerable.

How considerable an economy the swimbladder allows can be further evaluated by realizing that the drag of the water opposing a fish's movement is proportional to (velocity)$^{\frac{3}{2}}$ for laminar flow and approximately (velocity)^2 for turbulent flow (Kermack, 1948; Hill, 1950). Thus for some active pelagic fish a force of 7% of the body weight would enable a fish to *sustain continuously* a horizontal velocity of 43% or 53% (depending on whether the flow is laminar or turbulent) of that velocity which it would have if it exerted *continuously* a force of 25% of its body weight, a force fishes *seldom* exert. (If we take our reference velocity as that given by a fish exerting 50% of its body weight then 7% would give a sustained velocity of 27% or 37% of this velocity). The above calculations were made by E. J. Denton and T. I. Shaw.

The mackerel (*Scomber scombrus*) is one of the few freely swimming fishes from the neritic region without a swimbladder, and it is noticeable that it can only maintain its level in the water by restless and vigorous activity. This unceasing motion is perhaps the price paid for a facility of moving up and down quickly in the top layers of the sea; for to come quickly to the surface from 20 m would increase the volume of a swimbladder threefold, and, apart from the danger of internal damage, the fish would have to exert an upward force equal to about 10% of its weight in order to go down again.[1].

The 5% relation between the swimbladder and body volume is also found in bathypelagic teleosts (Kanwisher & Ebeling, 1957). This might suggest that the species without a swimbladder were faced with the same problems as the mackerel. But many of these fishes have fragile, lightly ossified skeletons, the scales are reduced or absent, and the muscle layers of the trunk and tail are thin. This must lead to some reduction of their weight in water and it has been suggested (Marshall, 1954, 1955) that they are not much heavier than their surroundings. Measurements by one of us (E.J.D) have shown this is certainly true of two fairly common bathypelagic fishes without a swimbladder, *Gonostoma elongatum* (order Isospondyli, suborder Stomiatoidea) and *Xenodermichthys copei* (order Isospondyli, suborder Clupeoidea). Analysis of their chemical composition has revealed how this reduction of specific gravity is achieved. However, in evading, or almost evading, the buoyancy problem, it is the muscles used in propulsion that are particularly reduced. Nevertheless, the proportion of muscle (as indicated by total protein) falls less than does the downward force which has to be exerted to keep the fish in a given horizontal plane. Because of loss of speed, this might, however, seem to be like 'jumping from the frying pan into the fire', but consideration of the biology and environment of the bathypelagic fishes without a swimbladder reveals much of how this problem seems to have been met.

MATERIAL AND METHODS

The buoyancy of the fish

These experiments were made in the Bay of Biscay aboard R.V. 'Sarsia'. The deep-sea fish were caught in an Isaacs-Kidd mid-water trawl, the live fish being put into sea water previously cooled to about 10° C. A piece of cotton was threaded through the lower jaw of a fish and the fish was weighed in air on a 100 g spring balance, and in sea water using a torsion balance of 1 g full-scale deflexion. The torsion balance was on a gimbal table and it proved possible to measure the weight of the fish in sea water to a few milligrams. Great care was taken for the weighing under sea water to have no bubbles of air on or inside the deep-sea fish and both spring balance and torsion balance were frequently checked with known weights. After checking the weight in sea water several times the fish was taken out of sea water, gently dried and placed in a dry honey jar or Kilner jar and stored in a deep-freeze. Before using the fish for chemical analysis the jar and fish were weighed and after removing the fish and drying the jar this was weighed alone. The difference between these two weights gave a check on the accuracy of the weighing of the fish which had been made at sea. At sea the temperature of the sea water used was noted and samples of the sea water were taken in sealed jars. The specific gravities of the samples were measured with a hydrometer on returning to Plymouth.

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[1] Dr G. Hughes (private communication) has noted that a considerable fraction of a fish's metabolism may be devoted to providing the flow of water across the gills. A fish like a mackerel which uses its forward motion to provide much of this flow may not be at such a disadvantage as simple calculation might suggest.

The common bathypelagic fish *Chauliodus sloanei* has a thick transparent gelatinous envelope around its body. It was thought that this might be less dense than sea water and thus provide some positive buoyancy. Pieces of this material cut away from the fish were, however, found to be heavier than sea water.

Direct measurements were made of the contribution of the swimbladder gases to the buoyancy of the coastal fish *Ctenolabrus rupestris*. Specimens of this fish, freshly killed, were weighed in air and in sea water after opening the fish, puncturing the swimbladder and squeezing out its gas. These measurements showed that such a fish without the gas in its swimbladder would have a weight in sea water of about 5·4 % of its weight in air. This weight must in very large part be attributed to the muscles (the largest single tissue component), for isolated pieces of muscle had in sea water about 5 % of their weight in air. Experiments on coastal fish of different species showed that the density of muscle varies appreciably; the weight of hake (*Merluccius merluccius*) muscle in sea water was only 3·2 % of its weight in air. These experiments suggested that a variation in the proportion of protein might be an important variable in the 'buoyancy balance sheet' of fishes. That this is so was borne out by the determinations of chemical composition described below.

Chemical analysis

The chemical analyses were made with the advice and help of Mr E. I. Butler. Not all fishes were analysed in the same way but the most complete analyses were made in the following way:

> The fish were ground with sand and anhydrous calcium sulphate and Soxhlet extracted with 40°–60° petroleum ether. The residue was reground and dried in an oven for several hours when further extracts were made until no further material could be extracted. The residue, insoluble in petroleum ether, was extracted in a Soxhlet apparatus with 96 % alcohol to constant weight of extract. The nitrogen in the final residue was estimated, and the alcohol extract refluxed with 40°–60° petroleum ether and filtered. The filtrate contained only that fraction of the animal's fat which was extractable by

alcohol but not petroleum ether and this was estimated by drying the filtrate to constant weight. The alcohol-soluble ether-insoluble nitrogen was estimated using the method of Kjeldahl.

As a check on this method the procedure was varied. On occasions total nitrogen was estimated using whole fish, whilst on other occasions the fat was extracted after grinding with sand fish which had been oven dried (105° C) to constant weight. The various methods used gave results which were in good agreement with one another. The dry weights given in this paper are those of fish cut into very small pieces and dried in an oven at 105° C to constant weight.

RESULTS

The buoyancy of Gonostoma *and* Xenodermichthys

All the fishes used were caught alive and in very good condition. The values for the (weight in sea water/weight in air) × 100 were for, 6 *Gonostoma* 0·54, 0·68, 0·90, 0·34, 0·40, 0·90 (average 0·63) and for 6 *Xenodermichthys* 1·4, 1·1, 1·4, 1·1, 1·2, 1·3 (average 1·25).

The sea water used for these buoyancy measurements was found to have a salinity close to that in which the fish live, i.e. about 35·5 g of salt in 1000 g of sea water. These fish are usually found down to 1000 m and, in the water of the Atlantic from which they were taken, there is little change in salinity between the surface and 1000 m (Cooper, 1952). The maximum error in the above figure for (weight in sea water/weight in air) which might be attributed to differences in salinity was 0·05 and this is disregarded.

Radiographs

The radiographs (Pls. I and II) show that the skeletons of *Chauliodus*, *Gonostoma* and *Xenodermichthys* are very poorly ossified in comparison with specimens of coastal fish *Gadus minutus* of comparable weight. The deep sea myctophid *Diaphus rafinesquei* which *has* a swimbladder is seen to have a skeleton as well ossified as that of a *Ctenolabrus rupestris* of about the same weight. Pl. II shows the remarkable difference in ossification between *Gonostoma elongatum* and *Gonostoma denudatum* both bathypelagic fish, the former without, and the latter with, a gas-filled swimbladder.

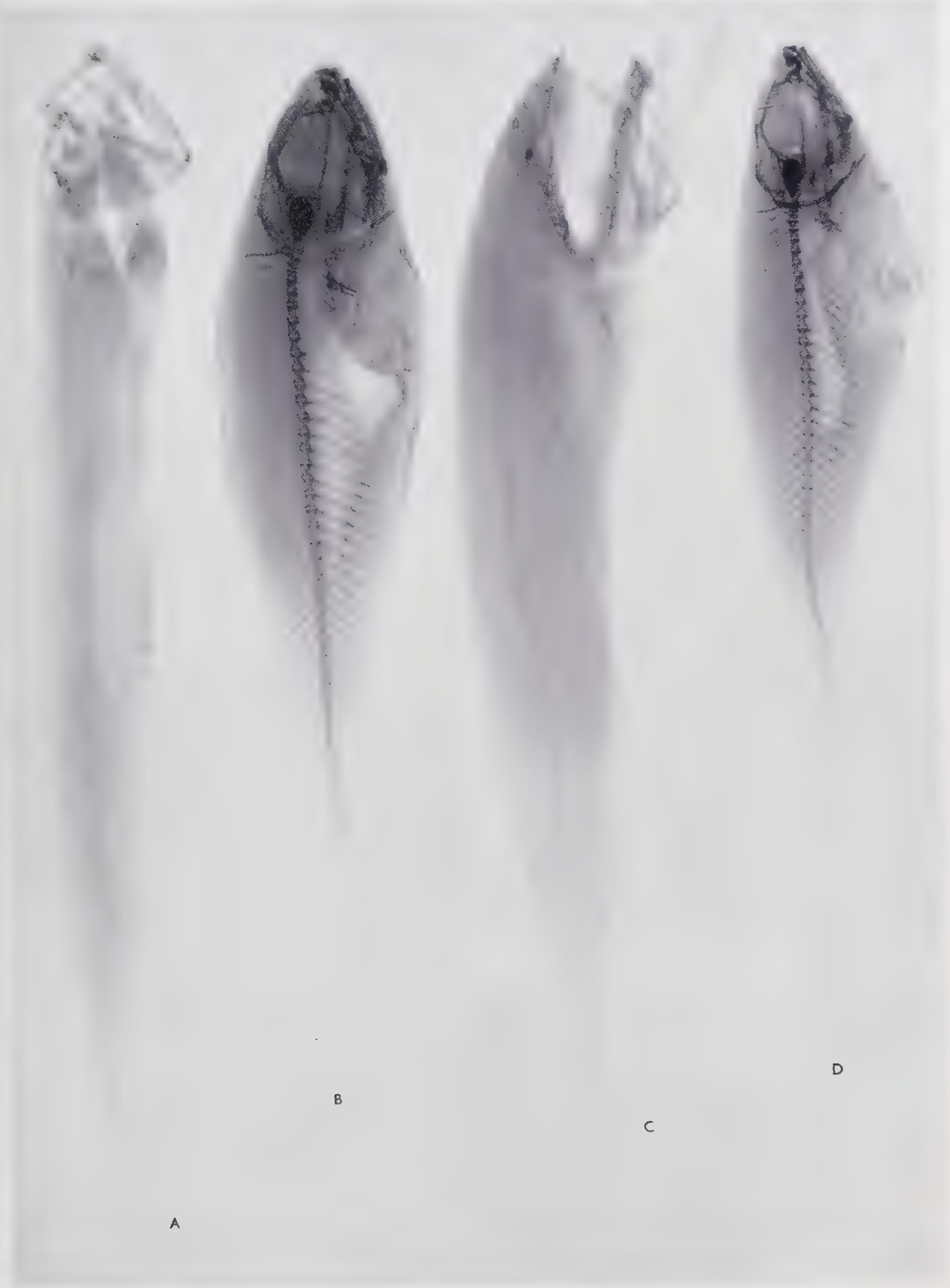
EXPLANATION OF PLATES

PLATE I

Radiographs comparing the degree of ossification of (A) *Chauliodus sloanei*, (B) *Gadus minutus*, (C) *Gonostoma elongatum*, and (D) *Gadus minutus*. Of the two specimens of *G. minutus* the second (D) is much smaller than either of the bathypelagic fish. The swimbladders of *G. minutus* appear as light areas in the body. Magnification × $\frac{4}{5}$.

PLATE II

Radiographs (above) showing the difference in ossification between (A) *Gonostoma bathyphilum* and (B) *G. elongatum*; and (below) comparing the degree of ossification of (C) *Ctenolabrus rupestris*, (D) *Diaphus rafinesquei*, (E) *Xenodermichthys copei*, and (F) *Gadus minutus*. Magnification × $\frac{4}{5}$.



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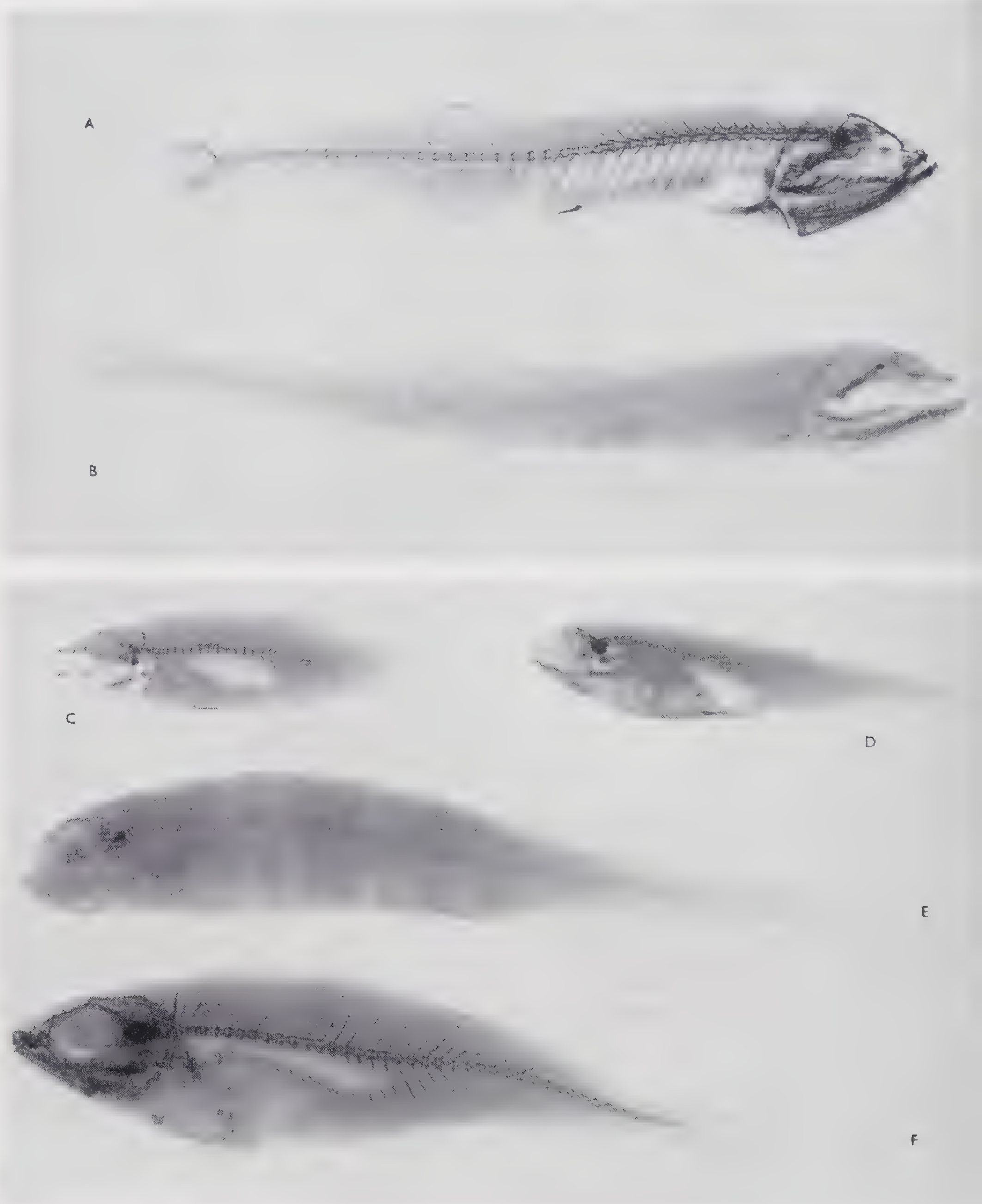
A
B
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The otoliths of the bathypelagic fish without swimbladders are very small when compared with those of the fish with swimbladders. Particularly striking is the contrast between the large otoliths of the small fish *Diaphus rafinesquei* with the tiny otoliths of *Chauliodus sloanei*.

Chemical analyses

The principal results of the chemical analyses are given in Table I. Components are given as percentage of wet weight. The protein is taken as being 6·025 × the total nitrogen (see Love, 1957).

TABLE 1. CHEMICAL ANALYSES

Fish	Fat extractable in 40°–60° petroleum ether	Total N_2	Protein	Dry weight	$\frac{\text{Wt. in sea water}}{\text{Wt. in air}} \times 100$
Gonostoma elongatum	2·6	—	—	—	0·68
„	5·3	—	—	—	—
„ *	3·4	0·71	4·3	12·6	0·34, 0·4
„	—	1·01	6·0	—	0·54
„	—	0·87	5·2	—	—
Xenodermichthys copei	0·51	—	—	—	—
„	0·55	—	—	—	—
„	—	1·2	7·2	—	—
„	—	—	—	9·8	—
„ *	—	1·2	7·2	11	1·1, 1·2
Ctenolabrus rupestris	—	2·76	16·6	—	—
„	—	—	—	28	—
„	—	—	—	26	—
„	0·5	—	—	—	—
Labrus bergylta†	—	2·72	16·4	—	—

* Two fish taken together. † These fish have a gas-filled swimbladder.

Part of the lipid material in fish is bound in such a way as not to be extractable in petroleum ether. This is a small fraction of the total lipid in fish which have a good deal of fat but an important fraction when there is little fat (Lovern, 1955). After extracting with petroleum ether some fish were therefore further extracted with 96% alcohol to constant weight of extract. This alcohol was shaken with petroleum ether and the residue from the ether, after evaporation, was taken as the ether inextractable fat. Part of the total nitrogen of the fishes was non-protein nitrogen and this was estimated on the alcohol extract after shaking it with petroleum ether. For one *Gonostoma* the extra fat corresponded to 0·5% of the wet weight and for two *Gonostoma* the non-protein nitrogen to 0·05 and 0·04% of the wet weight. For *Xenodermichthys* the extra fat corresponded to 0·7% of the wet weight and the non-protein nitrogen to 0·04% of the wet weight. These are figures which would give only very small corrections and they are not taken into account in the Discussion below.

DISCUSSION

Buoyancy properties

The buoyancy measurements were made at around 10° C which is close to the normal environmental temperature for these fishes. They were left for some time in sea water at this temperature before the measurements were made.

The measurements were made in the laboratory on R.V. 'Sarsia' with the fish under sea water at atmospheric pressure, but the fish are often caught at depths around 500 m where they are subject to pressures of around 50 atmospheres. This change in pressure can, however, make very little difference to the buoyancy of the fish, for the change in volume of water when the pressure is raised from 1 to 50 atmospheres is only one of about 0·2% and the volume of the fish will change in much the same way as does the sea water, leaving a residual change in buoyancy which is only a small fraction of 0·2%. We can therefore accept the surprising fact that *Gonostoma* is often within $\frac{1}{2}$% and *Xenodermichthys* within 1·2% of neutral buoyancy despite the fact that neither fish has a gas-filled swimbladder.

The chemical analyses show quite clearly how this is achieved. These are extremely watery fishes with poorly ossified skeletons. The dry weight of *Gonostoma* and *Xenodermichthys* are only 12·5 and 10% respectively of their weights, whereas the *Ctenolabrus rupestris*, used as a control, had a dry weight of about 28% of its wet weight. The fat content of the deep-sea fishes is not particularly high, averaging about 3%, but the protein content of about 5% of their dry weight is very low indeed when compared with the corresponding 16% for the typical coastal fishes, *Ctenolabrus rupestris* and *Labrus bergylta*.

Most of the data in the literature is for edible portions of fish (see Vinogradov, 1953, pp. 463–566). Shewan (1951) gives total nitrogen figures varying from 2·42 to 3·78% for skeletal muscles of many teleosts. These values may be compared with the 2·76–2·74 given here for whole wrasse used for control experiments. The protein content of the principal organs of higher vertebrates is shown in the *Handbook of Biological Data* (1956) as varying from about 10% for brain and spinal cord through about 20% for skeletal muscle to 30% for skin.

Marine fishes normally derive a considerable degree of buoyancy from the fact that their body fluids are considerably more dilute than sea water. The figures given by Krogh (1939) for the extracellular fluids of marine teleosts suggest that these have an approximate osmotic pressure of about 40% that of sea water. The intracellular fluids will be in osmotic equilibrium with the extracellular fluids, although the components will be different. The principal intracellular cation is probably potassium rather than sodium (this in itself will affect the density little), and most of the anions will be organic compounds such as the organic phosphates of muscle and the haemoglobin of red blood cells whose contribution to density has to some extent been included in the organic analysis. The intracellular concentration of chloride will almost certainly be very much lower than the extracellular concentration. We will, however, in the absence of good analyses, make the assumption that both the deep-sea fish and the wrasse have body fluids whose density lies half way between that of distilled water and sea water. [Preliminary analyses indicate that this cannot (in terms of density) be very seriously in error.] It is now possible to draw up balance sheets to explain the buoyancy properties of a deep-sea fish and a wrasse (Text-fig. 1, Tables 2 and 3). The negative weights imply that the component has a positive buoyancy in sea water.

TABLE 2. BALANCE SHEET FOR *GONOSTOMA ELONGATUM*

Component	% wet weight	Specific gravity	Weight in sea water/100 g of fish
Fat	3·7	0·91*	−0·5
Protein	5·0	1·33†	+1·1
Body fluids (water + dissolved salts)	87·6	1·013	−1·2
Other components including bone	3·2‡		+1·1‡

Buoyancy. These fish had no gas-filled swimbladder and their average weight in sea water was approximately +0·5 % of their weight in air. (Wet weight)

* *Handbook of Biological Data* (1956).
† Höber, (1954). Specific gravity taken as the reciprocal of the partial specific volume.
‡ These values are given by difference.

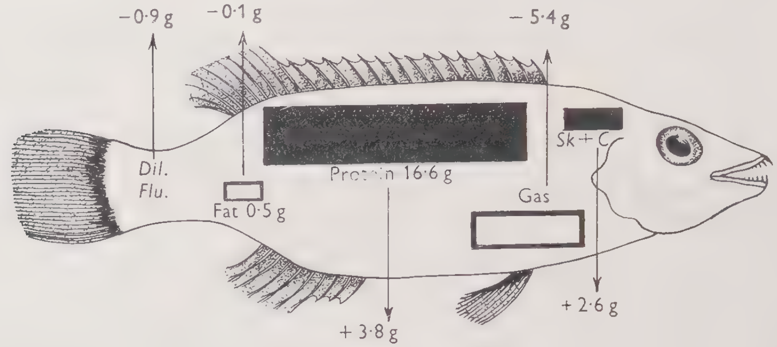
TABLE 3. BALANCE SHEET FOR *CTENOLABRUS RUPESTRIS*

Component	% wet weight	Weight in sea water/100 g of fish
Fat	0·5	−0·1
Protein	16·6	+3·8
Body fluids	73·3	−0·9
Other components including bone	9·2*	+2·6*

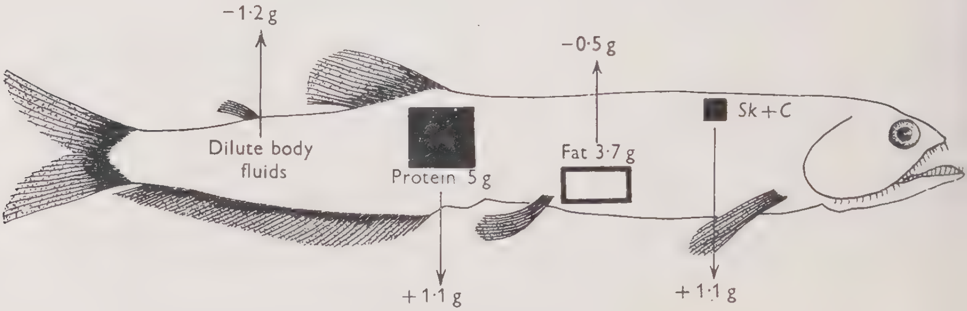
Buoyancy. This fish without its swimbladder has a weight in sea water of +5·4 % of its weight in air

* These values are given by difference.

Some idea of the contribution of the mineral components of bone is given by ashing the fish. Ashings in a platinum crucible of whole deep-sea fish (*Xenodermichthys copei*) gave for a high temperature (around 1000° C) an ash of 1·2% and for a low temperature (around 450° C) an ash of 1·9%. The corresponding figures for ash from wrasse (*Ctenolabrus rupestris*) were both 4·5%. The ash from wrasse contains large recognizable fragments; skull, vertebrae, etc., whilst that of the deep-sea fish is very much less in amount and much more powdery (Text-fig. 2). Some of the ash is sodium, potassium and chloride. Estimates made of these components on water extracts from the ashes indicate that the remaining mineral components account for 1·2% and 0·7% of the two deep-sea fish ashed. The densities of salts found in bone, e.g. apatite, (Harrow, 1954) are close to 3 (*International Critical Tables*, 1928) so that the contribution of this material to the buoyancy of the



Ctenolabrus rupestris



Gonostoma elongatum

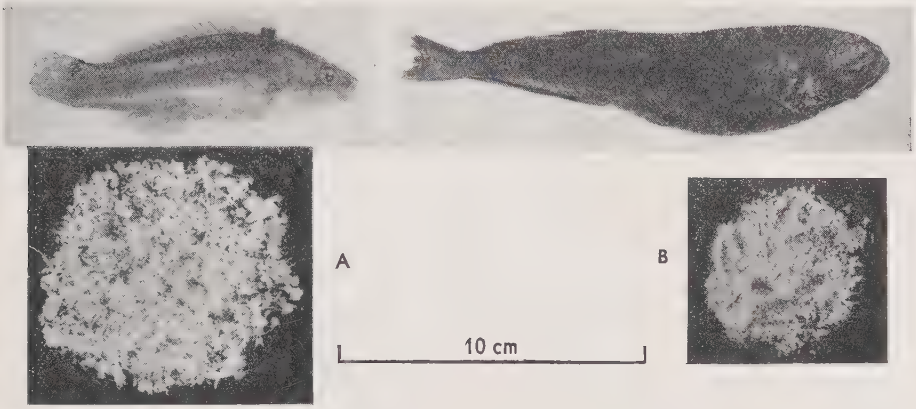
Text-fig. 1. Diagram of the 'buoyancy balance sheet' for a bathypelagic fish *Gonostoma elongatum* without a swimbladder (below) and a coastal fish *Ctenolabrus rupestris* with a swimbladder (above). Positive values are given for those components of the fish which are heavier than the sea water which they displace and thus tend to 'sink' the fish, whilst negative values are given for those components which displace more sea water than their own weight and thus tend to 'float' the fish. Weights given per 100 g of fish. *Dil. Flu.*, dilute body fluids; *Sk + C*, skeleton and other components.

fish will be about +0·6 g/100 g of fish for *Xenodermichthys*, which is a value rather smaller than that given in the above table on *Gonostoma* for 'other components including bone'.

General biological considerations

While these findings are striking in themselves, they may be seen in better perspective against a more general biological background.

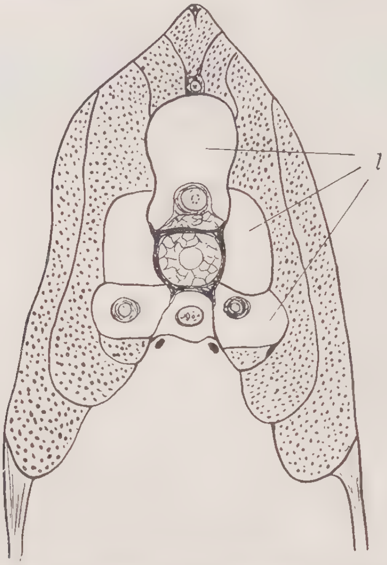
Bathypelagic fishes without a gas-filled swimbladder are quite diverse. The main groups in the order Isospondyli are Melanostomiatidae, Stomiatidae, Chauliodontidae, Idiacanthidae, Malacosteidae (stomiatoids), Alepocephalidae (clupeoids) and Bathylogidae (salmonoids). Of the order Iniomi, the entire suborder Alepisauroidea consists of fishes without any known trace of a gas-filled swimbladder at any stage of their life history (Marshall, 1955), and the same is true of the ceratioid angler-fishes (Bertelsen, 1951). Finally, there are the fishes of the small orders, Giganturoidea, Lyomeri, Cetunculi and Miripinnati, the last having a functional gas-filled swimbladder during the larval phase (Bertelsen & Marshall, 1956). These groups and a few not mentioned, make up nearly half of the bathypelagic fish fauna.



Text-fig. 2. A, a coastal fish with a swimbladder, *Ctenolabrus rupestris*, wet weight 16·7 g; with ash obtained from it shown below, weight 0·76 g. B, a deep-sea fish without swimbladder, *Xenodermichthys copei*, wet weight 31·2 g; with ash obtained from it shown below, weight 0·33 g.

In these fishes the nature and extent of the tissues appear to be very similar to the two species we have analysed in detail. The stomiatoid species certainly have lightly ossified skeletons (the scales are absent or poorly developed) and a correspondingly reduced musculature. Concerning the melanostomiatids and *Idiacanthus*, Beebe & Crane (1939) remark that '...as usual in deep-sea fish, the jaws are the only really strongly ossified parts of the body, the gill arches usually come next, then the tip of the caudal penduncle, while the skull proper, the rest of the vertebral column and the supports of the vertical fins are ossified very late, and then usually weakly.'

These observations also apply to the alepisauroid fishes (Marshall, 1955) and particularly to the Lyomeri, which have a persistent notochord and a very reduced skeleton (Tchernavin, 1947). The weak lateral muscles in one species (*Eurypharynx pelecanoides*) is well shown in transverse sections figured by Nusbaum-Hilarowicz (1923). The vertebral column is not immediately surrounded by the myotomes, but by extensive fluid-filled cavities, which appear to be lymphatic in nature (Text-fig. 3). The ceratioid angler-fishes also have weakly developed lateral muscles and lightly ossified skeleton (Bertelsen, 1951).



Text-fig. 3. Transverse section through the fore-part of the trunk of the gulper-eel, *Eurypharynx pelecanoides*, showing the extent of the muscle (dotted) and the lymphatic spaces (*l*). (After Nusbaum-Hilarowicz, 1923).

But, as we have already seen, such weakness of the skeletal and muscular systems is not true of bathypelagic fishes with capacious gas-filled swimbladders (for instance, the Myctophidae). Here we need only draw attention to the radiograph (Pl. II) of *Gonostoma elongatum* and *G. denudatum*. Earlier reference has been made to this (Marshall, 1954), but the plate gives more striking proof than words of the difference in ossification between these two related deep-sea fishes. The skeleton of *G. denudatum* (the outline of the swimbladder can also be seen) stands out sharply beside that of *G. elongatum*, in which the swimbladder is regressed and invested with fat. (It should be stressed that both fish are of adult size and that this difference between them can also be readily appreciated when they are handled).

While the bathypelagic fishes with a well developed, gas-filled swimbladder tend to be concentrated at depths above 1000 m, those lacking this organ are found at all mid-water levels known to contain fish. Of the groups listed above, most of the stomiatoids, bathylagids, alepisauroids, giganturoids and Miripinnati tend to occur above the 1000 m level, while the Lyomeri and ceratioid angler-fishes are mostly fished below this depth (for a review of the vertical distribution and references, see Marshall, (1954)). The latter seems to be also true of many alepocephalids,[1] but not of such forms as *Searsia* (Grey, 1956).

Turning now to the physical nature of their environment, most species of bathypelagic fishes are found in the tropical and temperate regions of the ocean between depths of about 250 m down to at least 3000 m. Owing to the rapid fall of temperature below the thermocline there is a corresponding increase in viscosity (between the above levels the laminar viscosity will be increased by a maximum factor of about 1·66). At the cooler, lower levels, a fish will thus gain more support from the surrounding water. If heavier than water it will sink more slowly, but it will find movements more difficult to make. At all events, it is interesting that the ceratioid angler-fishes, which appear to be little more than floating traps, make up most of the fish fauna at these lower levels.

Except for the Cetunculi and Miripinnati, which take small prey, particularly copepods (Bertelsen & Marshall, 1956), most of the bathypelagic fishes without a swimbladder consume a wide range of food organisms. Considering only their larger prey, the stomiatioid groups, the alepisauroids (excluding the paralepidids), the giganturoids, the Lyomeri and the ceratioid angler fishes are able to capture and master relatively large fishes, which may even be longer than themselves.

It is thus perfectly clear that these deep-sea predators are not handicapped by their relatively feeble muscular system. And, as we have seen, the main structures for holding and swallowing the prey, the jaws and gill arches, are supported by the most firmly ossified parts of the skeleton. Sections through the lower jaws of *Gonostoma elongatum* and *Diaphus rafinesquei* (a lantern fish with a capacious swimbladder) showed that the degree of ossification was much the same, but that the bone was thinner in the former. (This fish has a standard length of 205 mm and a lower jaw length of 36 mm, the bone of the latter having a maximum thickness of 0·03 mm. The corresponding measurements in the lantern fish are, 74, 16 and 0·12 mm). However, just beneath the tooth-bearing surface of the lower jaw and continuing to the angle, the bone in the gonostomatid has a honeycomb-like structure, which must give extra strength along the biting edge.

[1] Judging from trawl catches, a number of species appear to live close to the deep sea floor.

But while the jaws and associated structures have a relatively robust framework, the fish (or squid) has first to be caught. Observations on the capture of large prey have yet to be made, although both Mackintosh and Gunther (Clarke, 1950) watched a silvery stomiatoid fish attacking a swarm of krill at the surface. Gunther wrote: 'In its manner of lurking and snapping prey it resembled the freshwater pike.' Beebe & Crane (1939) observed that melanostomiatid fishes brought up alive '...swam about and snapped with all the accuracy of balance and swiftness of surface fish'.

However, fishes with thin lateral myotomes are hardly fitted to be tireless hunters, and this is borne out by their body forms and fin patterns. Like the pike, or better still the garfishes (Belonidae), the melanostomiatids, stomiatids and malacosteids have slim, elongated bodies and the dorsal and anal fins are opposed and set well back, close to the caudal fin. As in the species that Gunther saw, they are fishes which hover and dart after their prey. The combined dorsal, anal and caudal fins must form a powerful swimming organ (Beebe & Crane, 1939). Even more important, this fin arrangement will not only enable the fish to get away quickly from a hovering position but also confer stability during the dart (see Harris, 1952).

Dr S. Smith has noted from the radiographs that the myotomes are of relatively constant width along the length of the fish. We may compare the rapidly diminishing width of myotome on moving close to the tail of *Gadus minutus* with the almost constant width of myotome of the deep sea fish. The deep sea fish have not therefore flexible tails adapted for delicate movements.

The alepisauroids also tend to have an elongated pike-like form (the paralepidids are known as barracudinas), but only the anal fin has a posterior setting. One species, *Paralepis rissoi*, was observed from a bathyscaphe by Furnestin (1955), who describes it as hovering in a vertical position with the head or tail uppermost, apparently keeping its level by the flickering of the small dorsal fin. Then, from this position, the fish would suddenly 'jack-knife' and dart off. At least some of the other paralepidids may be expected to behave in a similar way, while a hovering-darting habit is likely to be common to all alepisauroids.[1]

The ceratioid angler-fishes achieve even greater economy of effort by luring their prey to within striking distance (Bertelsen, 1951). They are clearly floating traps *par excellence*. Brauer (1908) thought that the luminous chin barbels of stomiatoid fishes might also be used as lures, while in *Chauliodus* the long second ray of the dorsal fin, which is tipped with luminous tissue, also appears to be an angling device (Tchernavin, 1953). Some of the stomiatoids even have light organs within the mouth. It is also conceivable that the spongy luminous tissue on the tail of the gulper-eel, *Saccopharynx*, may also act as a lure.

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[1] The scopelarchids and evermannellids have upwardly directed, tubular eyes and thus might be expected to adopt a horizontal position when hovering.

Lastly, it should be emphasized that these fishes live in the quieter parts of the ocean under the thermocline, which has an average depth of about 75 m. Above the thermocline the currents are relatively fast and wind-driven; below this level the (density) currents are more sluggish and the water is much less turbulent. In these deeper reaches a fish with relatively reduced propulsive powers can use these to greater effect (during a sudden dart) than under more boisterous conditions.

To summarize, bathypelagic fishes without a swimbladder often radically reduce their weight in water by developing lightly ossified skeletons, which are associated with reduced muscular systems, particularly along the trunk and tail.[1] In spite of this many of them are able to capture and swallow large prey. However we must add that this tendency is not invariable among such predacious fishes. Certain of the Astronesthidae and *Chiasmodon* (the great swallower) have well developed swimbladders (together with firm skeletons and fairly thick lateral myotomes). A closer comparison between these bathypelagic fishes and those without a swimbaldder would clearly be interesting.

We should like to thank Mr G. Parish for advice and help with the radiography, and Captain C. A. Hoodless and the crew of R.V. 'Sarsia' for their co-operation. We are grateful to Mr R. G. Maddock for excellent technical assistance.

SUMMARY

Gonostoma elongatum is often within 0·5%, and *Xenodermichthys copei* within 1·2%, of neutral buoyancy despite the fact that neither of these fish has a gas-filled swimbladder.

The dry weights of *Gonostoma elongatum* and *Xenodermichthys copei* are only about 12·6 and 10% of their wet weights compared with 28% for a typical coastal marine fish *Ctenolabrus rupestris*.

The fat content of *Gonostoma elongatum* and *Xenodermichthys copei* is not particularly high, averaging about 3% of their wet weight, but their protein content which is only about 4–7% of their wet weight is very low indeed when compared with the corresponding 16% for a typical coastal marine fish.

Radiographs of *Gonostoma elongatum*, *Xenodermichthys copei* and *Chauliodus sloanei* show that their skeletons are poorly ossified when compared with that of *Gadus minutus* a typical coastal marine fish of the same size, whereas the skeleton of *Diaphus rafinesquii* is ossified as well as that of *Ctenolabrus rupestris* a coastal marine fish of similar size. The skeleton of *Gonostoma*

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[1] It is difficult to imagine a much weaker skeleton without much weaker muscles for, as A. V. Hill (1950) writes: 'Athletic animals in fact, have rather a small factor of safety...if a man's muscles could be altered without altering his general design, so as to allow him to run 25% faster or jump 50% higher, athletics would become a highly dangerous pastime; pulled tendons, torn muscles, even damaged bones, would be so frequent as to make it prohibitive.'

denudatum, a bathypelagic fish with a swimbladder, is shown to be very well ossified in contrast with that of the related species *Gonostoma elongatum*.

The ash of *Xenodermichthys* was found to be about 1·5% of the wet body weight, whilst that of *Ctenolabrus rupestris* was about 4·5%.

The 'design' of these fish in relation to the life they lead is discussed. The muscular system is particularly reduced along the trunk and tail; whilst the jaws and gill arches, the main structures for holding and swallowing their prey, are supported by the most firmly ossified parts of the skeleton. These fish are almost certainly not tireless hunters, but rely on quick darts, often using luminous lures to attract their prey. Many of them could be simply described as floating traps.

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OBSERVATIONS ON THE LITTORAL ALGAE OF THE ISLE OF WIGHT

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(Text-figs. 1–4)

As a result of the geological formation of the Isle of Wight there is a great variety in the type of shore. Although much of the coastline is unsuitable for the establishment of algal communities there are some parts where a few species can exist, and others where full zonation is achieved. It therefore seemed worth making some study of the algal distribution. Towards this end a general survey was made of the south coast of the island in April 1955, with more detailed studies of the places of interest by means of transects in April and July 1955.

Previous observations on the algal flora include a list of records given by Morey (1909), and additions to it made by Delf & Grubb (1923) and Norkett (1947). In the present investigation the nomenclature used is that given in the Check Lists of Parke (1953) and of Hendey (1954). The observations presented are limited to the most common algae.

The behaviour of the tide around the Isle of Wight is unusual. In the English Channel the shallow water produces a quarter-diurnal harmonic curve superimposed on the semi-diurnal. From Swanage to the Nab Tower the phases are in a relationship such that a double or prolonged high water is produced. In the Isle of Wight the effect is strongest in the west, where also the tidal range is lowest. At Freshwater there is a distinct double high water at springs but a single prolonged one at neaps. In the east, at Bembridge, the effect is hardly apparent, high water at springs being only slightly prolonged. As low water is always about 6 h after the first high water in these areas, it follows that the ebb, once it has begun, is faster than the flood.

GEOLOGICAL STRUCTURE

The main geological regions of the Isle of Wight are shown in Fig. 1. The southern half of the island is composed of Cretaceous beds affected by two anticlines which run roughly east and west and overlap west of the centre. The uppermost beds, which are of Chalk, rise steeply as a ridge across the centre of the island. The Chalk thus extends to the coast at the Needles and at Culver Cliff. On the south-west coast the Upper, Middle and Lower Chalk are followed south-eastwards by Upper Greensand, Gault, Lower Greensand

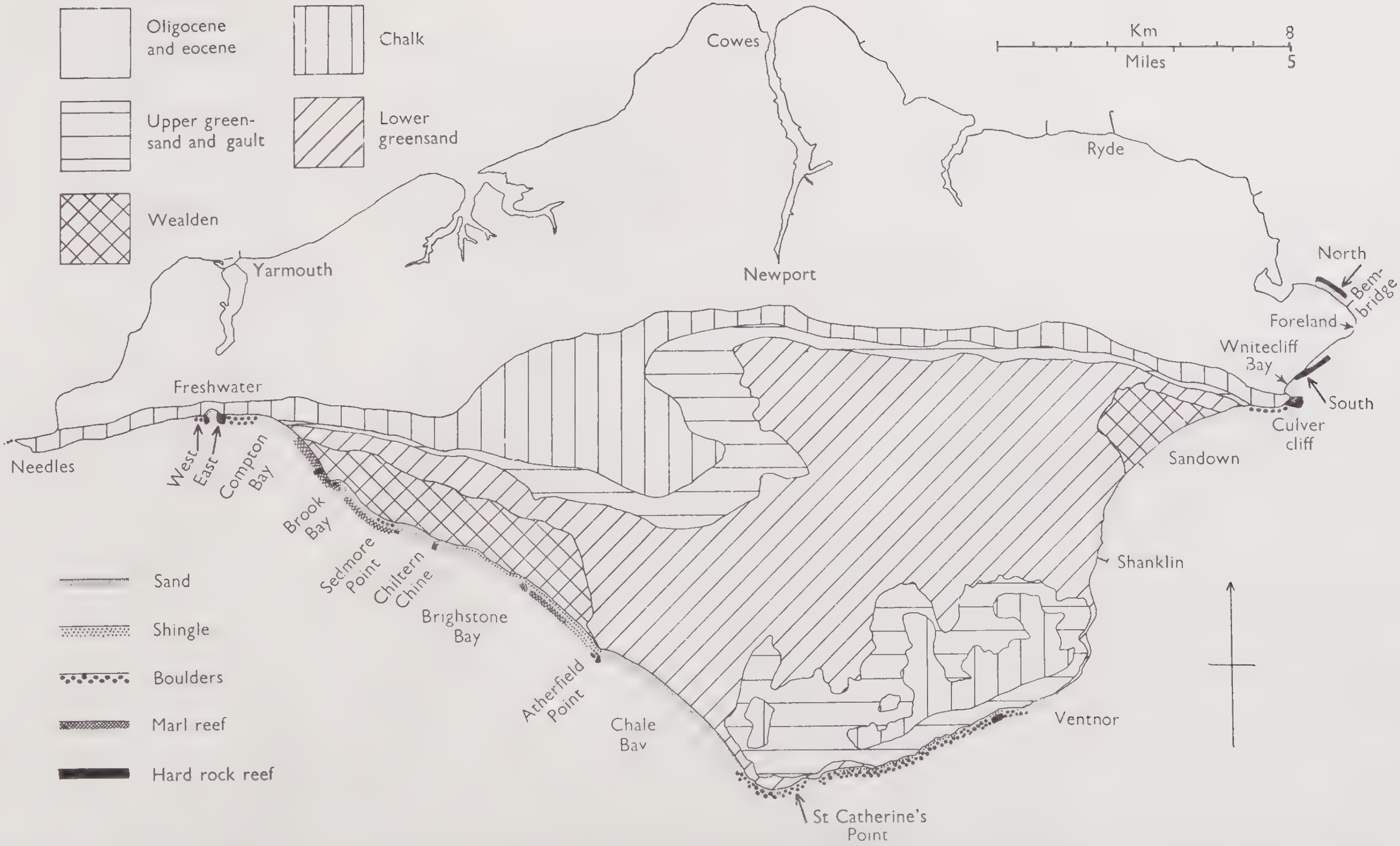
Oligocene and eocene
Chalk
Upper green-sand and gault
Lower greensand
Wealden
Sand
Shingle
Boulders
Marl reef
Hard rock reef
Km
Miles
8
5
Cowes
Newport
Ryde
Yarmouth
Freshwater
Needles
West
East
Compton Bay
Brook Bay
Sedmore Point
Chiltern Chine
Brighstone Bay
Atherfield Point
Chale Bay
St Catherine's Point
Ventnor
Shanklin
Sandown
Culver cliff
South
Whitecliff Bay
Foreland
Bem-bridge
North

Fig. 1. Map of the Isle of Wight showing the geological structure (taken mainly from Chatwin, 1948) and the nature of the shore along the south coast. The arrows indicate the positions of transects.

and Wealden, the lowest beds being exposed at about Sedmore Point. The series then ascends to St Catherine's Point. The south-east coast shows a similar sequence passing north-eastwards, the lowest beds being exposed in Sandown Bay. The southern limb of the anticline has a gentle slope, and where the Chalk reappears inland of St Catherine's Point the beds are almost horizontal. The soft underlying Gault has resulted in large sections of these Downs slipping down.

GENERAL SURVEY

A general survey of the type of shore and the distribution of the principal algae was made by walking along the shore and noting the substrata and the species present. Only the southern coast was studied. The northern shores are mostly of mud or sand, with some boulders in places.

From the Needles to Freshwater the cliffs of hard Upper Chalk drop almost sheer down to the sea. On either side of Freshwater Bay there are hard Chalk reefs which support fairly rich algal communities. These were studied with the use of transects (p. 775). The Middle and Lower Chalk cliffs of Compton Bay have boulders at their bases. Below the narrow belts of Upper Greensand, Gault and Lower Greensand the shore is sand or shingle. From the centre of Compton Bay to Sedmore Point most of the shore is occupied by a grey and purple Wealden Marl reef. This rock is smooth and very soft, but a number of algae are able to colonize it. The most common are *Polysiphonia nigrescens*, *Corallina officinalis* and *Cladostephus spongiosus*. *Cladophora*, *Fucus vesiculosus*, *F. serratus*, *Ceramium rubrum*, *Ulva lactuca*, *Rhodochorton* and some diatoms are also present. From Sedmore Point to the centre of Brighstone Bay is mostly sand except for a hard reef opposite Chiltern Chine. This supports a flora similar to that on the Marl, with the exception of *Polysiphonia nigrescens*, but with the addition of *Lomentaria articulata*, *Chondrus crispus*, *Rhodymenia palmata*, *Laurencia pinnatifida*, *Furcellaria fastigiata* and *Ptilota plumosa*. The Marl reefs of Brighstone Bay are for the most part covered with sand and support no algae. The hard reef off Atherfield Point was not accessible when it was visited but clearly supported fucoids. Chale Bay has a beach of fine sand and shingle.

Between St Catherine's Point and Ventnor the shore is strewn with boulders, sometimes forming a complete covering and sometimes mainly in the bays, in patches alternating with sand or shingle. There is usually a band of shingle at the top of the beach just below the cliff. The boulders support fairly extensive algal communities. The more detailed study of St Catherine's Point was of a fairly representative section (p. 775).

The shore between Ventnor and Shanklin was not visited. From there to the beginning of Culver Cliff, where the Chalk reappears on the shore, is sandy. Boulders lie at the base of the cliff and a Chalk reef extends from it. This supports a fairly extensive flora. Just north of the sand of Whitecliff Bay the ledges of Bembridge Limestone around the Foreland begin. These are more extensive on the south side than the north, where there is a greater proportion of sand. Both places were studied in more detail (p. 776).

Of the 25 miles of coast visited, 9 miles have hard reefs or boulders and 3 miles have Marl reefs. The rest of the coast is sandy.

SHORE TRANSECTS

Five shores were selected for more detailed study by means of transects: the Chalk boulders west of and the Chalk reef east of Freshwater Bay, the boulders below St Catherine's Point lighthouse, some limestone reefs north of Whitecliff Bay ('Bembridge South') and limestone reefs at Bembridge Point ('Bembridge North'). These are marked in Fig. 1.

Levelling apparatus

A simple form of levelling apparatus was developed for the use of the single-handed worker. As it is very cheap to assemble it will be described in some detail.

The sighting level is shown in Fig. 2A–D. It consists of a carpenter's level with wooden sides added to support a partly silvered piece of glass (to reflect the bubble) in the centre, a fine wire horizontally across one end and a brass plate, with a pinhole, across the other end. In order to adjust the sighting line between the hole and the wire so that it is parallel to the level itself, the brass plate is moved up or down until readings of height taken in opposite directions with the instrument are identical.

It is usual with this type of instrument to have it at a fixed height and to read the height on a staff in the position to be measured. But as this involves another worker holding the staff the proceeding has been reversed: the staff is fixed in one position with a clear triangular marker, and the heights of the positions to be measured are determined by sliding the instrument up or down a graduated movable staff until it is level with the marker and then reading its height on the movable staff.

The fixed staff is shown in Fig. 2E, F. It is constructed mainly of 0·6 m lengths of 1·2 × 2 cm wood bolted together in such a way that it can be folded to a length of 0·7 m. The staff is 2·1 m high and is marked at 0·5 m intervals from the ground. The pointer can be attached at any of the four marks by means of a bulldog clip. The staff is held upright by two legs, each a metre long and with a hinge at the top end which can be bolted through any of a number of alternative holes. It can thus be set up on uneven ground. The bottom ends of the legs and the staff itself have strap hinges with flat pieces of leather attached. Stones can be placed on these so that the whole is stable and rigid. The movable staff is shown in Fig. 2G, H. It is marked in centimetres.

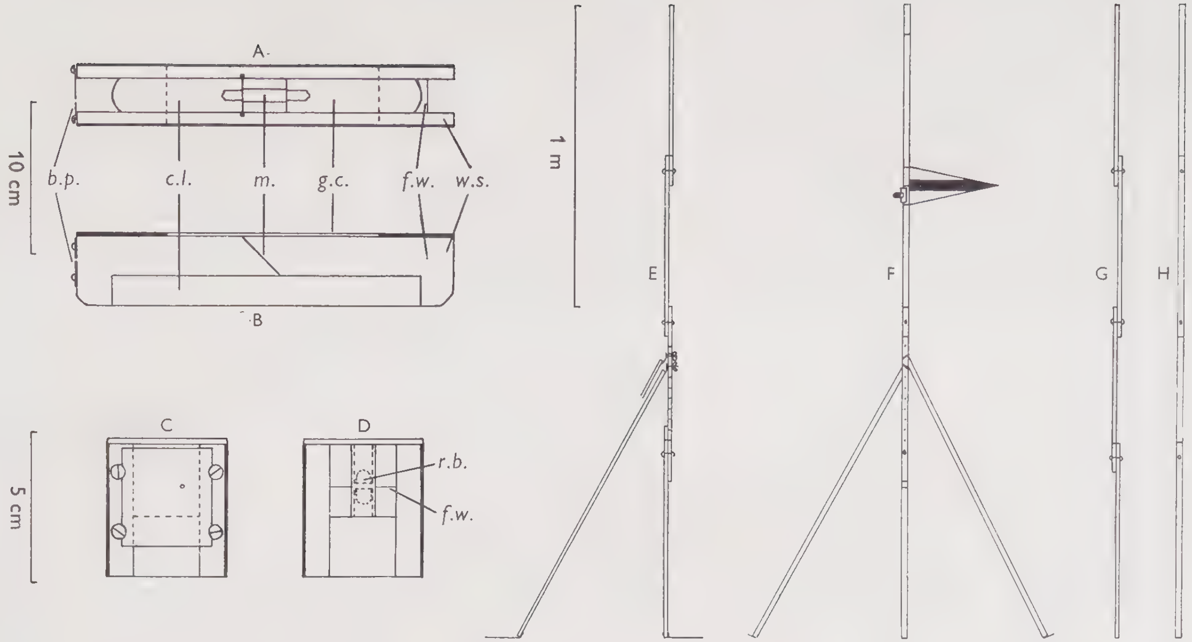

Fig. 2. Diagrams of the sighting level (A–D), fixed staff (E and F) and movable staff (G and H). A, top view of level; B, side view; C, end view with brass plate in place; D, end view with plate removed; E, side view of fixed staff; F, front view; G, side view of movable staff; H, front view. *b.p.*, brass plate; *c.l.*, carpenter's level; *f.w.*, fine wire; *g.c.*, glass cover; *m*, mirror; *r.b.*, reflexion of bubble; *w.s.* wooden sides.

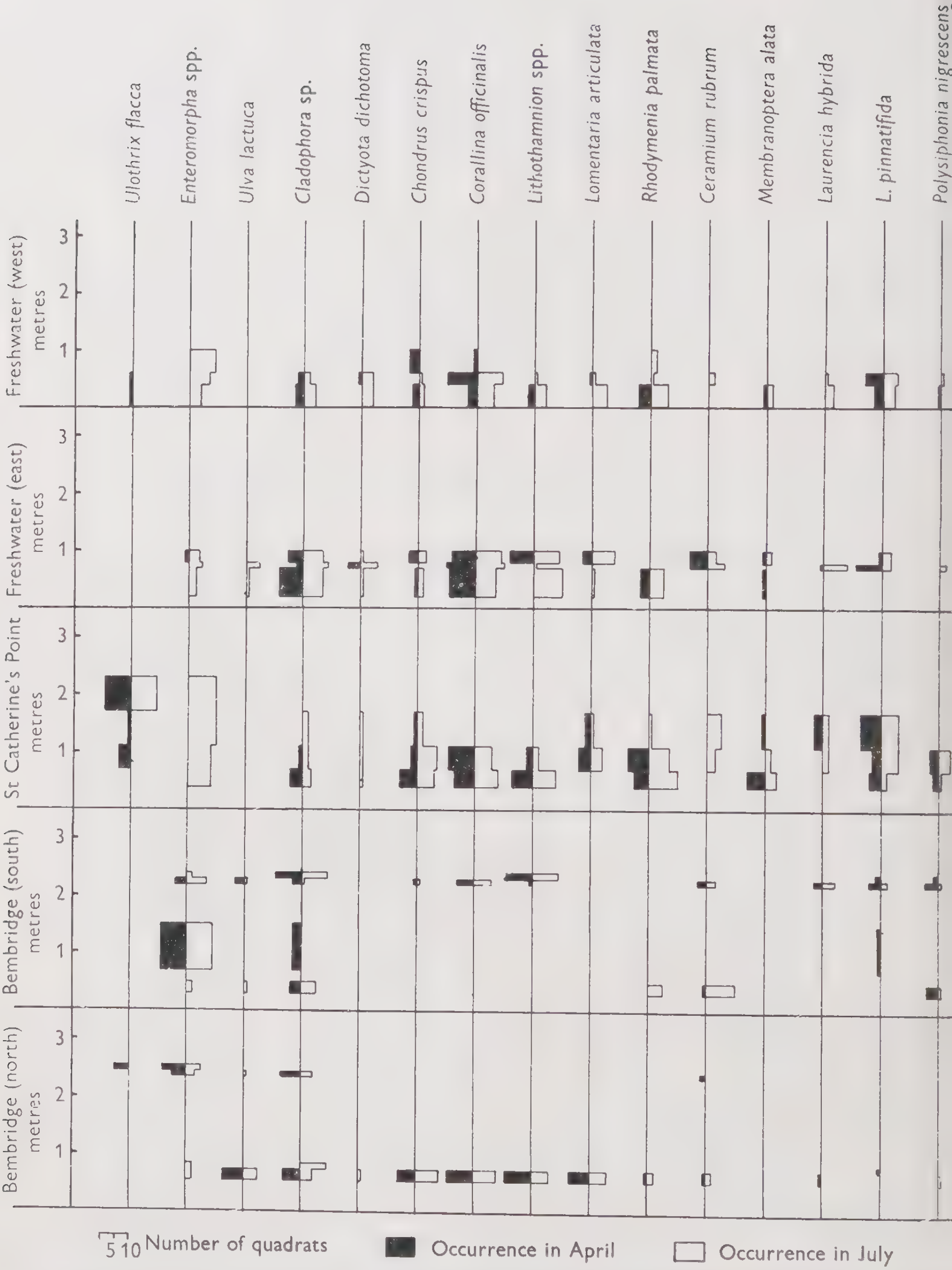

Fig. 3. Diagram of the occurrence of the most important species in quadrats at various levels in the fiv localities, in April and July.

Method of survey

A marked line was run out down the selected part of the shore. The level of each major irregularity was determined and sketches made of the profile. The limits of the main algal communities were noted and at about the centre of each a 10 m line was placed at right angles to the profile line. At each metre on this line a 25^2 cm quadrat was placed and the species present in it noted. The limits of the *Fucus* and *Laminaria* were determined by taking the level of the highest and lowest (if accessible) plant in each metre-wide band for 5 m on one side of the profile line.

The heights were referred to chart datum by measuring the level of low water and calculating its height from the predicted low water for the locality corrected for the deviation on that particular day, shown by the tide gauge at Portsmouth.

The original survey was made in April. In July the same line was visited again, quadrats taken in the same places to the nearest metre, and the *Fucus* and *Laminaria* zones similarly noted.

Observations

The nature of the transect method resulted in a very limited area of a particular shore being studied. Although it was made as representative as possible it clearly cannot be taken as a description of the whole shore.

A total of sixty-five macroscopic and twenty-five diatom species occurred in the quadrats, in April and July together. There were more brown and red species in July than in April, and this was the true for all the five locations taken individually.

The most important species, other than *Fucus* and *Laminaria* spp., are shown in Fig. 3. The levels over which they extended are approximate and were determined from the levels (given by the profile diagram) of the limits of the communities noted on the shore. It is clear that *Enteromorpha* was very much more abundant in July than April. *Dictyota dichotoma*, *Ceramium rubrum* and possibly *Laurencia hybrida* were also more abundant in July. In addition to these *Leathesia difformis*, *Cystoclonium purpureum*, *Ceramium ciliatum* and *C. diaphanum* occurred in a number of quadrats only in July. The distribution of the other species shown was similar in July to that in April, especially that of *Corallina officinalis* and *Lithothamnion*.

At Freshwater the height to which the species could extend seems to have been limited by the nature of the substratum, which was sheer cliff above 1 m. The exposed nature of the shore probably rendered this uninhabitable. The species therefore all had similar ranges. At St Catherine's Point the higher boulders were occupied by *Enteromorpha* and *Ulothrix*, other species extending up to about mid-tide. At Bembridge (South) it may have been the flat slow-draining ledge which enabled many species to extend to 2·2 m above chart datum, whereas the same species were limited to 1·7 m at St Catherine's Point where the slope was steep and drainage fast. At Bembridge (North) only a few species existed on the ledge at about 2·5 m, the rest being limited to a lower ledge below a belt of sand.

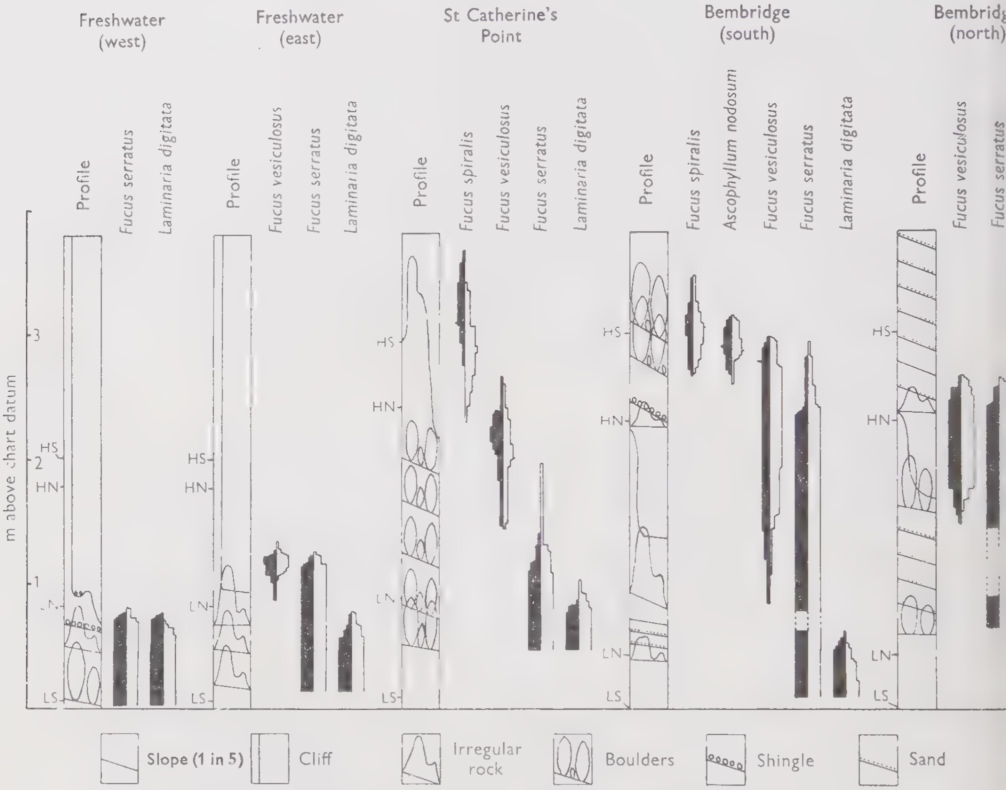
Freshwater (west)
Freshwater (east)
St Catherine's Point
Bembridge (south)
Bembrid (north)
Profile
Fucus serratus
Laminaria digitata
Profile
Fucus vesiculosus
Fucus serratus
Laminaria digitata
Profile
Fucus spiralis
Fucus vesiculosus
Fucus serratus
Laminaria digitata
Profile
Fucus spiralis
Ascophyllum nodosum
Fucus vesiculosus
Fucus serratus
Laminaria digitata
Profile
Fucus vesiculosus
Fucus serratus
m above chart datum
3
2
1
HS
HN
LN
LS
Slope (1 in 5)
Cliff
Irregular rock
Boulders
Shingle
Sand

Fig. 4. Diagram of the summarized profiles in the five localities, together with the limits to which the fucoids and *Laminaria* extended, in April and July. HS, HN, mean high water springs and neaps; LN, LS, mean low water neaps and springs. See text for further explanation.

The fucoid and *Laminaria* zones are shown in Fig. 4. Included with them are the diagrams summarizing the profile of each shore. These were constructed from profile drawings in which the horizontal scale was a fifth of the vertical. The slope is thus much steeper than in reality. Each line representing the slope on the diagram refers to the region above the line, and the type of shore in that region is indicated diagrammatically.

The existence of *Fucus spiralis* at Freshwater was probably precluded by the exposed nature of the vertical cliff at the level it would have occupied. It was present on another part of the shore where there were boulders at this level. On the east side *F. vesiculosus* occupied the tops of the rock irregularities, while the flatter parts were dominated by *F. serratus*. At St Catherine's Point four zones were present, but the shore was apparently too exposed and steep for *Ascophyllum*. The steepness was presumably responsible for preventing *Fucus serratus* from extending higher, as it did on the gentle slopes of the Bembridge shores. At Bembridge (South) *Ascophyllum* was also present on the flat reef, but *Fucus vesiculosus* extended farther down the steeper part, where the surf was probably too strong for *Ascophyllum*. Here the upper limit of *Laminaria* was lower than at the other places, as a belt of sand prevented its extension upwards. Sand also severely limited zone-formation on the northern Bembridge shore, *Fucus vesiculosus* and *F. serratus* occupying the upper ledge.

A complete list of the species encountered is given in Tables 1 and 2 (pp. 779–80). The frequencies in the quadrats of the macroscopic forms are given (Table 1).

Finally it must be said that no definite conclusions can be reached on the factors determining the distribution on these shores without more prolonged observations or experiments. It is possible that similar studies made after several years might reveal important differences in the distribution of many species.

My thanks are due to Mr A. C. Kain for the design and construction of the levelling apparatus and to the Hydrographer (Admiralty) and the Assistant Queen's Harbour Master, Portsmouth, for tidal information. I am also indebted to Dr E. M. Burrows, Dr P. J. Dixon, the late Dr K. M. Drew Baker, Dr N. I. Hendey, Dr and Mrs D. E. G. Irvine and Dr M. W. Parke for the identification of various algae. Dr Hendey was entirely responsible for the identification of the diatoms given in Table 2, and I am extremely grateful to him.

SUMMARY

The southern coast of the Isle of Wight, though mainly sandy, is composed in parts of marl reefs bearing a few algae and in other parts of boulders or hard reefs which bear full algal zonation.

Transect and quadrat studies showed that in 1955 there were more species present in July than April and several species showed a marked increase in cover in July. The vertical distribution of the common species showed little change.

The levels or existence of the fucoid zones are dependent on the slope of the substratum.

A levelling technique suitable for single-handed work is described.

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APPENDIX

TABLE 1. LIST OF ALGAE

The species (other than diatoms) recorded in this work at Freshwater West (F.W.-W.), Freshwater ast (F.W.-E), from Compton Bay to Brighstone Bay (S.W.), at St Catherine's Point (St. Cath.), from Catherine's Point to Ventnor (S.E.), at Bembridge South (Bemb.-S.) and at Bembridge North (Bemb.-.) in April and July. The occurrence in quadrats is expressed as a percentage of the total number of uadrats taken at that place.

	F.W.-W.		F.W.-E.		S.W.	St Cath.		S.E.	Bemb.-S.		Bemb.-N.	
	Apr.	July	Apr.	July	Apr.	Apr.	July	Apr.	Apr.	July	Apr.	July
umber of quadrats...	30	30	30	30		40	40		35	35	50	50
ostera nana	—	—	—	—	—	—	—	—	—	—	4	—
lidingia minima	—	—	—	—	—	—	—	—	—	—	—	2
haetomorpha aerea	—	—	—	—	—	23	—	—	—	—	—	—
ladophora spp.	17	20	50	77	+	13	18	+	54	34	30	44
odium tomentosum	—	—	—	—	—	—	—	—	3	6	+	2
nteromorpha spp.	+	67	3	40	+	—	90	+	37	54	10	14
lothrix flacca	—	—	—	—	—	35	23	—	—	—	10	—
lva lactuca	6	—	—	20	+	—	—	+	9	6	16	18
scophyllum nodosum	—	—	—	—	—	—	—	+	+	3	—	—
sperococcus fistulosus	—	—	—	—	—	—	—	—	6	—	2	10
horda filum	—	—	—	—	—	—	—	—	—	—	—	6
ladostephus spongiosus	3	7	—	3	+	10	3	+	—	6	—	—
olpomenia peregrina	—	—	—	—	—	—	—	—	—	—	14	—
ictyota dichotoma	3	27	13	20	—	—	8	—	—	—	—	6
ucus serratus	13	20	40	40	+	28	28	+	49	43	22	20
spiralis	—	—	—	—	—	+	+	+	+	+	+	—
vesiculosus	—	—	+	+	+	5	8	+	6	3	4	6
alidrys siliquosa	—	—	—	—	—	—	—	—	6	3	2	—
alopteris scoparia	—	—	—	—	—	—	—	—	—	—	—	4
aminaria digitata	7	10	+	20	—	20	23	+	+	+	4	10
saccharina	—	—	—	3	—	—	3	—	—	—	6	4
eathesia difformis	—	13	—	30	—	—	—	—	—	6	—	—
etalonia fascia	—	—	—	—	—	—	—	+	3	—	—	12
ylaiella littoralis	—	—	—	—	—	—	5	—	11	—	8	—
phacelaria fusca	—	—	—	—	—	—	—	—	—	6	—	—
radicans	—	—	—	—	—	—	—	—	—	6	—	—
hnfeltia plicata	—	—	3	3	—	—	3	—	—	—	—	—
allithamnion hookeri	—	—	—	—	—	5	—	—	—	—	—	—
. tetricum	—	—	—	—	—	—	3	—	—	—	—	—
eramium ciliatum	—	20	—	20	—	—	—	—	—	—	—	—
deslongschampsii	—	—	—	—	—	—	—	+	—	—	—	—
. diaphanum	—	3	—	7	—	—	5	—	—	—	—	—
. flabelligerum	—	—	—	—	—	3	5	—	—	—	—	—
. rubrum	—	7	7	30	+	—	20	+	11	23	16	12
hondrus crispus	17	10	13	17	+	23	38	+	3	3	24	34
orallina officinalis	47	50	90	87	+	40	35	+	17	17	30	34
ystoclonium purpureum	—	7	—	—	—	—	5	—	—	—	—	4
umontia incrassata	—	—	—	13	—	5	—	—	3	—	12	—
urcellaria fastigiata	3	—	7	7	+	—	—	—	6	—	7	6
astroclonium ovatum	—	7	3	—	—	—	8	—	—	—	—	—
elidium coinale	—	—	—	—	—	—	—	—	—	—	—	2
. latifolium	—	—	—	—	—	—	—	—	—	—	2	4
igartina stellata	—	—	—	—	—	—	—	+	3	—	—	—
riffithsia flosculosa	—	—	—	—	—	—	—	—	—	—	2	4
alopitys incurvus	—	—	—	—	—	—	—	—	3	—	12	6
nia rubens	—	—	—	—	—	—	—	—	—	—	2	10
aurencia hybrida	—	13	3	27	—	8	10	+	9	11	2	—
pinnatifida	30	30	33	20	+	35	35	+	20	6	4	4
thothamnion spp.	7	17	60	67	—	23	25	+	29	26	36	28
omentaria articulata	3	20	10	30	+	15	13	+	—	—	20	24
embranoptera alata	3	7	7	7	+	20	13	+	—	—	2	10
yllophora membranifolia	—	—	13	20	—	—	—	—	6	—	14	10
ocamium coccineum	—	—	13	7	—	—	3	—	—	—	14	20
lyides caprinus	—	—	—	—	—	—	—	—	11	11	—	—
lysiphonia denudata	—	—	—	—	—	—	—	—	—	—	—	4

TABLE 1 (*cont.*)

	F.W.-W.		F.W.-E.		S.W.	St Cath.		S.E.	Bemb.-S.		Bemb.-N	
	Apr.	July	Apr.	July	Apr.	Apr.	July	Apr.	Apr.	July	Apr.	Ju
P. fibrata	–	–	3	–	–	–	–	–	–	–	–	–
P. lanosa	–	–	–	–	–	–	–	+	–	3	–	–
P. nigrescens	3	3	–	7	+	13	13	+	29	3	–	
P. urceolata	–	–	–	–	–	–	–	+	–	3	–	–
Porphyra umbilicalis	–	–	–	–	–	–	–	+	–	–	–	–
Pterosiphonia thuyoides	–	7	+	–	+	–	–	–	–	3	–	2
Ptilota plumosa	–	–	–	–	+	–	5	–	–	–	–	–
Rhodochorton floridulum	–	–	–	–	+	–	–	+	–	–	4	6
Rhodomela confervoides	–	–	–	–	–	–	–	–	–	–	–	2
Rhodymenia palmata	13	30	10	17	+	30	45	+	+	11	14	22
Spermothamnion barbatum	–	–	20	–	–	–	–	–	–	–	–	–
Spyridia filamentosa	–	–	–	–	–	–	–	–	–	–	–	1

TABLE 2. LIST OF DIATOMS

The diatom species recorded in this work at Freshwater West (F.W.-W.), Freshwater East (F.W.- from Compton Bay to Brighstone Bay (S.W.), from St Catherine's Point to Ventnor (S.E.) and at Be bridge South (Bemb.-S.) in April.

	F.W.-W.	F.W.-E.	S.W.	S.E.	Bemb.-
Actinoptychus senarius	+	+	–	–	–
Amphipleura rutilans	–	+	–	+	–
Amphora arenicola	–	–	–	+	–
A. exigua	+	–	–	–	–
Biddulphia alternans	+	–	–	–	–
B. aurita	+	+	+	+	–
B. mobiliensis	–	–	–	+	–
Cocconeis costata	+	–	+	–	–
C. dirupta	–	–	+	–	–
C. scutellum	–	–	–	+	–
C. scutellum var. *stauroneiformis*	–	–	–	+	–
Coscinodiscus excentricus	+	–	–	+	–
Diploneis bombus	+	–	–	+	–
Grammatophora oceanica	–	–	+	–	–
Licmophora gracilis	+	–	+	+	–
L. lyngbyei	+	–	–	–	–
Mastogloia binotata	–	–	+	–	–
Navicula cluthensis	–	–	–	+	–
N. cyprinus	–	–	–	+	–
N. distans	+	–	–	+	–
N. grevillei	+	+	–	+	+
N. mollis	+	–	+	+	+
N. ramosissima	+	+	+	+	+
Nitzschia acuminata	–	–	–	+	–
N. angularis	+	–	–	+	–
N. lanceolata	–	–	–	+	–
N. panduriformis	–	–	–	+	–
N. punctata	+	–	–	+	–
N. punctata var. *coarctata*	–	–	–	+	–
N. sigma var. *rigidula*	+	–	–	+	+
Paralia sulcata	+	–	–	+	–
Podosira stelliger	–	–	–	+	–
Rhabdonema arcuatum	–	–	+	–	–
R. minutum	–	–	+	–	–
Rhaphoneis amphiceros	+	–	–	+	–
R. surirella	–	–	–	+	–
Rhoicosphenia curvata	+	–	–	+	–
Rhopalodia musculus	–	–	–	+	–
Synedra affinis	+	–	+	+	–
S. gaillonii	–	–	+	+	–
S. investiens	+	–	+	+	–
S. tabulata	–	–	–	+	–
Thalassiosira decipiens	+	–	–	+	–
Trachyneis aspera	+	–	–	–	–

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STUDIES ON THE GROWTH OF MARINE PHYTOPLANKTON

II. *ISOCHRYSIS GALBANA* PARKE

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(Text-figs. 1–8)

A good deal is known about the growth in culture of *Phaeodactylum tricornutum* Bohlin (*Nitzschia closterium* W. Sm. forma *minutissima* Allen & Nelson) (see Spencer, 1954; Harvey, 1955; Provasoli, McLaughlin & Droop, 1957). This organism, however, is a somewhat aberrant member of the Chrysophyta, and other marine and brackish-water representatives of the group, e.g. *Syracosphaera carterae* Braarud & Fagerland (Braarud & Fagerland, 1946; Provasoli, McLaughlin & Pintner, 1954), *Prymnesium parvum* Carter (Reich & Kahn, 1954; Droop, 1954), and certain other species (Droop, 1954, 1955*a*, *b*) have been studied much less intensively from this point of view. It has therefore seemed worth while making a general study, similar to that reported in the first paper of this series for the diatom *Asterionella japonica* Cleve & Müller ex Gran, of the growth requirements of a representative of the Chrysophyceae. *Isochrysis galbana* Parke, a flagellate of some importance as a food organism of the oyster, has been selected for this purpose. Johnston (1955) has used unialgal cultures of this species in studies of dissolved organic matter in sea water and, since the investigation being described was carried out, Droop (1957) has reported that it has a requirement for cobalamin.

MATERIALS AND METHODS

The culture of *Isochrysis* used was isolated by Dr M. W. Parke from Port Erin fish ponds in 1938 (Parke, 1949). It was obtained from the Plymouth collection shortly after it had been partly freed of bacteria by phototaxis. It was not purified further.

The methods used for the culture and study of this organism were almost the same as those used for *Asterionella* (see Kain & Fogg, 1958). In stagnant culture a glass bead was included in each boiling tube to help in the removal of cells adhering to the bottom. Media were autoclaved at 15 lb. for less than 1 min instead of for 15 min. The media used were identical to those used for *Asterionella*, omitting sodium silicate and the buffer. They are denoted AO

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(similar to AK for *Asterionella*), AQ_N (similar to AR_N) and AQ_A (similar to AR_A). AO and AQ_N, containing natural sea water and soil extract in the case of AO, presumably contained cobalamin though it cannot be certain that the amount of this was always sufficient to maintain the optimum growth of *Isochrysis*. Vitamins were sometimes added to AQ_A.

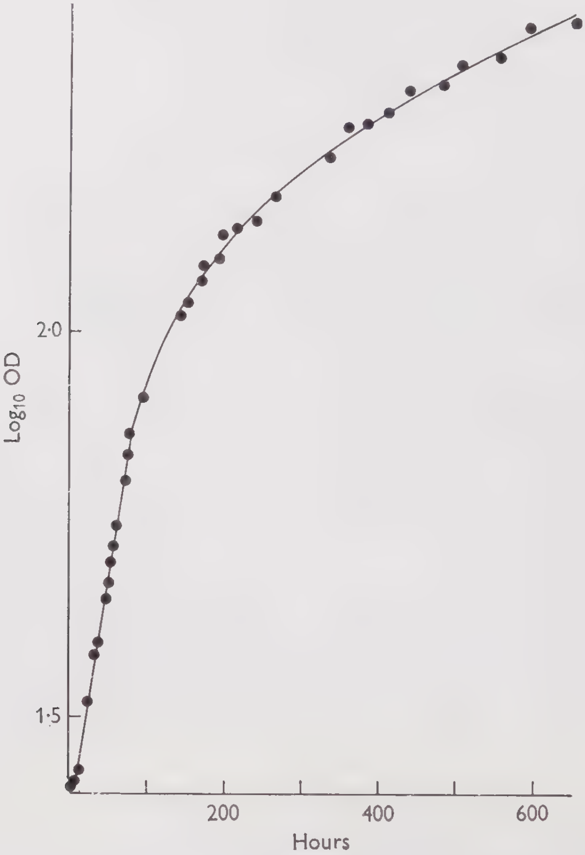

Fig. 1. The mean growth curve, determined by optical density readings, of two cultures of *Isochrysis*.

EXPERIMENTS

Growth

An example of the growth curve of *Isochrysis* in stagnant Erdschreiber medium is shown in Fig. 1. There was normally a lag period of up to 24 h after which growth was exponential, with a relative growth constant (defined as $(\log_e N_t - \log_e N_0)/t$ in days) of $k = 0{\cdot}25$ to $0{\cdot}55$. After 100–150 h growth was limited by the availability of carbon dioxide but continued slowly for up to 500 h, giving final populations of the order of 10,000 cells/mm^3. During the growth of *Isochrysis* in stagnant culture the pH of the medium rose to a higher level than for *Asterionella*, pH 9 being exceeded after 400 h.

Nitrogen supply

Isochrysis was grown in a series of concentrations of potassium nitrate in the artificial sea water medium (AQ_A), from an inoculum that had been washed in nitrate-free artificial sea water. Cell counts were made after 700 h and the cell crop in each culture calculated by subtracting the cell concentration in the controls to which no nitrate had been added from the cell concentration in the culture itself. This is shown plotted against the initial nitrate concentration in Fig. 2, which also gives the quantity of nitrate added to the medium divided by the crop obtained from it. The two lowest concentrations were evidently limiting and in these the mean nitrogen content was 0·0507 $\mu\mu$g-atom N/cell ($0{\cdot}7 \times 10^{-9}$ mg N/cell).

Phosphorus supply

An analogous experiment on the phosphorus requirement was made with a series of concentrations of dipotassium hydrogen phosphate. The results are shown in Fig. 3. The mean quantity of phosphorus in the cells in the lowest three concentrations, where it was limiting, was 0·000972 $\mu\mu$g-atom P/cell ($0{\cdot}03 \times 10^{-9}$ mg P/cell).

Salinity

The relative growth constant of *Isochrysis* in natural sea water was reduced to 0·27 at 10‰ S but showed no statistically significant deviation from a mean value of 0·36 over the range 15–40‰ S.

Artificial sea water

In medium AQ_A, composed principally of the major constituents of sea water and the trace elements with EDTA as used by Provasoli *et al.* (1957), the growth of *Isochrysis* was normal only when soil extract was present. This may be at least partly explained by its requirement for cobalamin. Unpublished work by Miss S. Arregger has shown that *Isochrysis* grows well in a synthetic medium, S50 of Droop (1958), in which 0·25 g/l. of glycylglycine seems to be an important component.

Temperature

The relative growth constants at different temperatures in medium AO, expressed as percentages of the mean of those of control cultures at 25° C, are plotted in Fig. 4. Between 20° and 25° C was optimal; at 30° C no growth

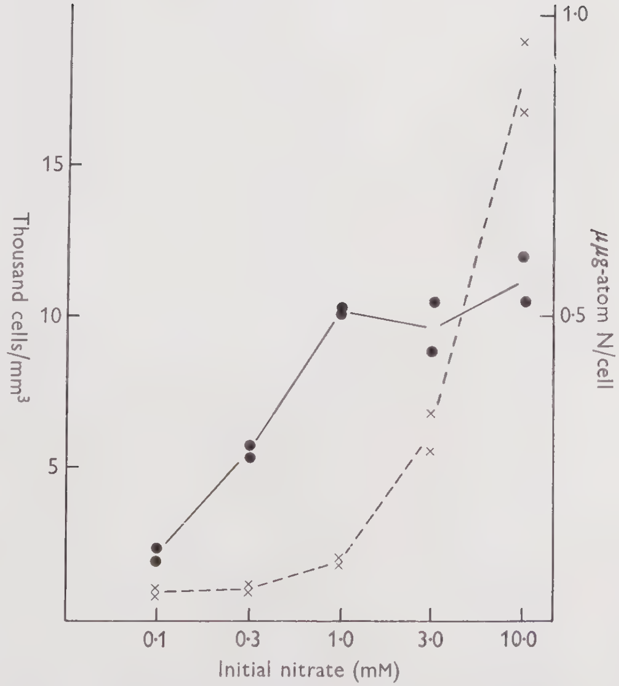

Fig. 2. The cell crop and nitrogen per cell of *Isochrysis* in relation to concentration of potassium nitrate. –●–●–, cell crop; – – × – – – × – –, nitrogen/cell.

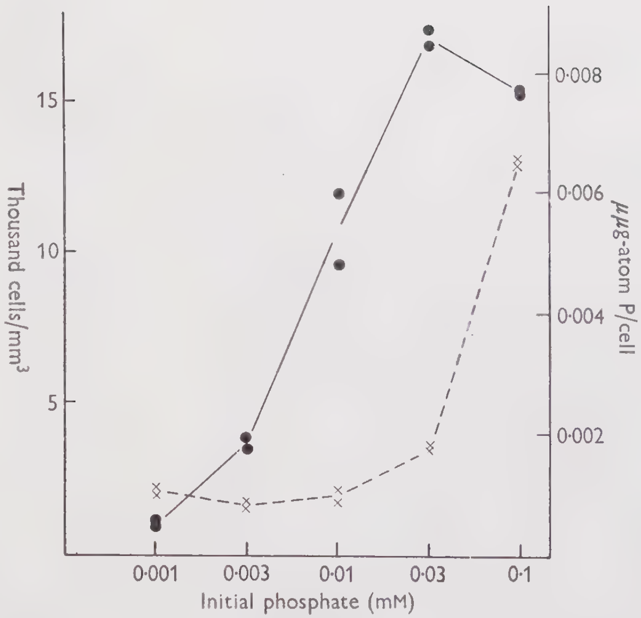

Fig. 3. The cell crop and phosphorus per cell of *Isochrysis* in relation to concentration of dipotassium hydrogen phosphate. –●–●–, cell crop; – – × – – – × – –, phosphorus/cell.

took place, but exposure for a week to this temperature was not lethal as cultures returned to 20° C were able to grow.

Light

The results of two experiments to determine the relative growth constants at various light intensities in medium AQ_N are shown in Fig. 5. Saturation was reached at about 1500 lux, an intensity of the same order as that saturating for the growth of *Chlorella pyrenoidosa* (Myers, 1953); there was no inhibition at 3000 lux. In both experiments the optical density per cell at the end of the exponential phase was considerably lower at higher than at lower light intensities.

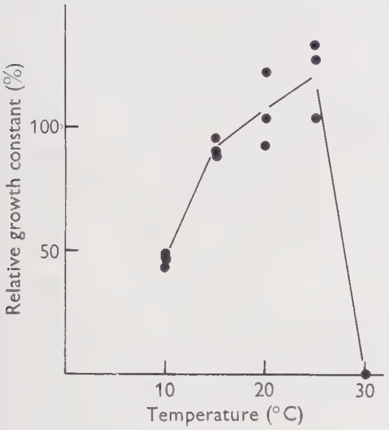

Fig. 4

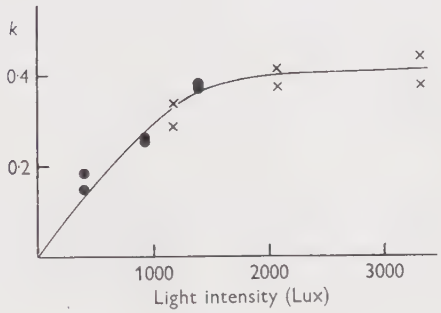

Fig. 5

Fig. 4. The relative growth constant, as % of controls, of *Isochrysis* at different temperatures. (See text for further explanation.)

Fig. 5. The relative growth constant (k) of *Isochrysis* in different intensities of fluorescent light. ●, Expt. 1; ×, Expt. 2.

Aeration

The mean growth curves of triplicate aerated and stagnant cultures in medium AQ_A are shown in Fig. 6. The mean relative growth constant was higher under aerated ($0·526 \pm 0·025$) than under stagnant ($0·446 \pm 0·026$) conditions, but the difference between the values was scarcely statistically significant at the 5% level. It is clear, however, that growth at the initial rate was continued to a much higher cell concentration (about 14,000 cells/mm^3) under aerated than under stagnant conditions (about 6000 cells/mm^3). A final population of 24,000 cells/mm^3 was attained in aerated culture on occasion.

Hydrogen-ion concentration

In determining the optimum pH for the growth of *Isochrysis* it was not possible to use the buffer tris(hydroxymethyl)aminomethane at a really effective concentration as this was inhibitory (see below). Instead 1·65 mM of tris was used and daily adjustments made to the pH of the medium (AQ_A), by means of the dummy tube method previously described (Kain & Fogg,

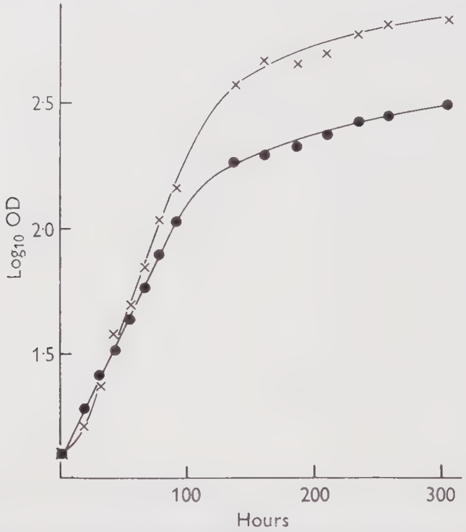

Fig. 6. The growth curves, determined from optical density readings, of *Isochrysis* in stagnant and aerated culture. -●—●-, stagnant; --×---×--, aerated.

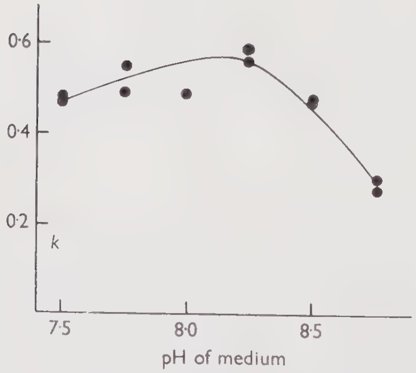

Fig. 7

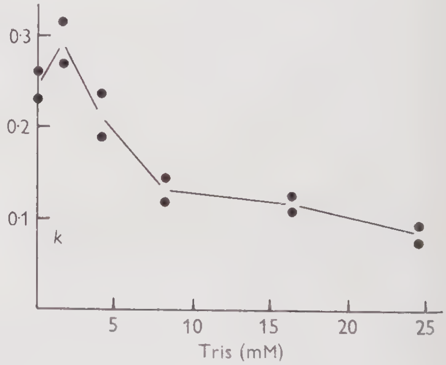

Fig. 8

Fig. 7. The relative growth constant (*k*) of *Isochrysis* in media adjusted to various pH's.

Fig. 8. The relative growth constant (*k*) of *Isochrysis* in relation to concentration of the buffer tris.

1958). The maximum variation observed was 0·3 pH units at pH 7·5. It was mostly about 0·1 pH unit. The pH of each experimental tube was determined at the end of the exponential phase and found not to differ by more than 0·12 pH units from that of the corresponding dummy tube. The relative growth constants are shown in Fig. 7. There was clear inhibition at pH 8·75 only.

The relative growth constants in a range of tris concentrations are shown in Fig. 8. There was a marked inhibition at 8·25 mM and above. The difference between the *k* values for 1·65 mM and for the controls is not statistically significant. In a further experiment using triplicate cultures *k* values of 0·446 ± 0·026 and 0·446 ± 0·017 were obtained for control and 1·65 mM tris cultures respectively. It therefore appears that low concentrations of tris have no appreciable stimulatory effect on *Isochrysis*. The marked inhibition of the growth of *Isochrysis* in 8·25 mM of tris shows that this organism differs from those tried by Provasoli *et al.* (1957) which were all tolerant of 0·1% (8·25 mM) of the buffer.

These results will be discussed together with those for other species on a later occasion.

This work has been carried out under extra-mural contract with the Institute of Seaweed Research. We are grateful to the Institute for a maintenance grant made to one of us (J. M. K.). We are also grateful to Dr M. W. Parke for the culture of *Isochrysis* and for helpful suggestions.

SUMMARY

The flagellate *Isochrysis galbana* has been grown in unialgal culture under controlled conditions and its growth has been measured by means of optical density determinations and cell counts.

The relative growth constant has been found to be 0·55 $\log_e$ units per day under optimum conditions. Waters with salinities from 15 to 40‰ S supported rapid growth. The optimum temperature was 20° to 25° C and the optimum light intensity from 1500 to at least 3000 lux. The rate of growth was inhibited at pH 8·75 and above. The buffer tris(hydroxymethyl)aminomethane had an inhibitory effect at concentrations of 8 mM and above. Final yields of the order of 24,000 cells/mm^3 could be obtained in cultures aerated by bubbling air. The minimum nitrogen requirement per cell was 0·0507 $\mu\mu$g-atom N/cell and the minimum phosphorus 0·000972 $\mu\mu$g-atom P/cell.

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NEUROMUSCULAR ACTIVITY IN SEA ANEMONE *CALLIACTIS PARASITICA* (COUCH)

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(With Text-figs. 1–7)

In a recent study (Ross, 1957) isolated marginal sphincter preparations of the sea anemone, *Calliactis parasitica*, showed two distinct responses to stimulation: (1) a quick contraction in response to stimuli at frequencies from 0·2 to 3·0 sec, the facilitated response of Pantin (1935*a*) beginning only on the second stimulus of a series, with a latent period of about 0·1 sec; (2) a smooth slow contraction in response to stimuli at lower frequencies, with a latent period of not less than 30 sec, and beginning only after several stimuli. This slow movement of the marginal region had not been detected in work on whole animals, but it is similar to the contractions of the 'slow' muscles of *Calliactis* (Pantin, 1935*b*) and *Metridium* (Batham & Pantin, 1950*a*). However, *Calliactis* sphincter shows almost no spontaneous activity.

Both quick and slow contractions had previously been observed in excised sphincters and mesenteric retractors of *Metridium* (Batham & Pantin, 1954), the chief muscles utilized in the closure of that animal. In all these cases, the quick contraction would seem to be a specialized mechanism for sudden withdrawal superimposed upon, and perhaps developed from, a more primitive and general slow contractile mechanism. As part of a programme to investigate and compare the properties of quick and slow contractions in these muscles it seemed desirable to see what part, if any, slow contractions of the marginal sphincter play in the life of *Calliactis*. This involved us in a wider study of the neuromuscular activity of this anemone which normally lives on shells inhabited by the hermit crab, *Eupagurus bernhardus*. We report the results here as an additional contribution to the analysis of behaviour in the Actinozoa, supplementing the similar studies on *Metridium* by Batham & Pantin (1950*a*, *b*).

METHODS

As in the work of Batham & Pantin (1950*b*, 1954), this investigation required the use of several methods of recording and observing the movements of the animals. Kymograph records, together with visual and photographic records, were used for information on the frequency, size and form of certain periodic activities. A short film record supplemented these observations. Preparations of *Calliactis* consisting of several rings joined to a strip of column were studied for information on innate rhythmic activity and on the timing and co-ordination of movements at different levels of the column. Responses to electrical and other forms of stimulation were also studied, both in whole animals and preparations, in order to see how they resembled the natural movements without stimulation.

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RESULTS

With its thicker and tougher integument and mesogloea, *Calliactis* does not show the extremes of shape and posture observed in *Metridium*. It can close down to a flattish cone but it never assumes the wrinkled and shrivelled appearance that is seen so commonly in *Metridium*. It can extend itself or bend to the side only to moderate degrees, and local contractions of the circular muscle of the column never produce in *Calliactis* the conspicuous deep furrows so clearly seen in *Metridium* (Batham & Pantin, 1950*a*). Moreover, unlike *Metridium*, *Calliactis* does not 'walk'; once it becomes firmly fixed on a plate or a stone, it may remain in the same place for months. It only moves by detaching itself altogether and creeping or rolling about the tank in a prone position.

Close observation of *Calliactis* shows, as Batham & Pantin (1950*b*) have remarked, that this anemone, like *Metridium*, is in a state of continual activity. Although most of the movement is too slow to be visible and the changes in appearance are less conspicuous than in *Metridium*, shape and posture are often quite different from one minute to the next. Moreover, there are periods when an exceptional amount of activity seems to be taking place and these periods are repeated at regular intervals.

Besides slowly changing in posture and shape, *Calliactis* closes up from time to time, even in the absence of any obvious stimulus. In order to find out how often it does so, ten animals were observed closely for 5 min periods at hourly intervals, ten times every day for a week. This gave us a record of the number of closing movements in 350 min observation time. In fourteen of the seventy observation periods, all ten animals remained open the whole time. The total number of closures recorded was 108, and on two occasions, four different animals exhibited closing movements during the whole or part of the same 5 min period. There were big differences in individuals. One was closed twenty-one times; another was never seen to close during the seventy observation periods. Thus closure may occur in some animals several times an hour, but in the majority it occurs with a frequency of about once an hour, and in some it is a very rare event indeed.

Two kinds of closing movement were evident in these animals. Some, like the responses to mechanical and electrical stimulation, were of short duration, with little loss of water or change of volume and the animal expanded again within a minute or two (Pantin, 1940; Chapman, 1949). Others were long-lasting. Animals closed at the beginning were frequently still closed at the end of an observation period, and some of these were also closed at the next and even later observation periods as well. From occasional checks in between, we know that in many of these cases no opening occurred in the intervening periods. These closures, which may be sustained for several hours, comprised about one-third of the total, and since the contraction of the animal in these closures was more complete and water loss greater, relaxation and opening were very much slower than in the familiar closing responses of short duration.

Since one of the objects of the investigation was to find out if slow contractions of the sphincter play any part in the normal behaviour of *Calliactis*, the closing movements were followed closely to see if slow contractions of the margin could be detected in them. It may be significant in this connexion that the first stages of these closing movements were not often seen. Closures beginning with a quick contraction were spotted only seven times. In the remaining 67 closures, the animals were already partly closed before any movement was detected. This may mean either that an observer tends to miss an initial quick contraction or that the movement begins slowly and only becomes apparent later. Subsequently, smooth flowing movements of the margin could be seen taking part in completing the closing movement, though occasionally the jerky movements of a quick contraction might be inserted into the sequence. Relaxation after many of these closures and partial closures was slow, resembling the time-course of relaxation after slow contractions of sphincter preparations (Ross, 1957) rather than the dramatic opening which follows quick closures. We concluded, therefore, that slow contractions of the sphincter contribute substantially to the spontaneous closing movements that occur from time to time in unstimulated *Calliactis*.

Closing movements are not the only feature of the behaviour of unstimulated *Calliactis*. The margin is frequently thrown into graceful folds by local contractions in certain radii. Changes in length and local constriction and peristalsis in the column produce a variety of shapes, some of which are illustrated in Fig. 6. Defaecation, with its gaping mouth and shapeless appearance, is easily recognized in *Calliactis* as in *Metridium* (Batham & Pantin, 1950). Many of these phases involve considerable shortening of the margin without quick sphincter contractions. Thus slow movements of the sphincter seem to be involved in these activities also.

RHYTHMIC CYCLES OF ACTIVITY IN *CALLIACTIS*

Some of the movements seen in *Calliactis* over a period seemed to have a repetitive character. Kymograph recordings gave clear evidence of this, and often showed a slow rhythm of remarkable regularity. Fig. 1 is a 3 hr record of *Calliactis* attached to a light gimbal lever. The big downward movements represent slow contractions of the submarginal and marginal regions culminating in a shortening of the column. This took 3–4 min to complete and was repeated every 10–15 min. The regularity, frequency and size of the main movement in the cycle vary greatly from one animal to another, and in the same animal at different times. But repetitive movements are always a feature of the records and they usually have marginal and submarginal components which are repeated several times per hour.

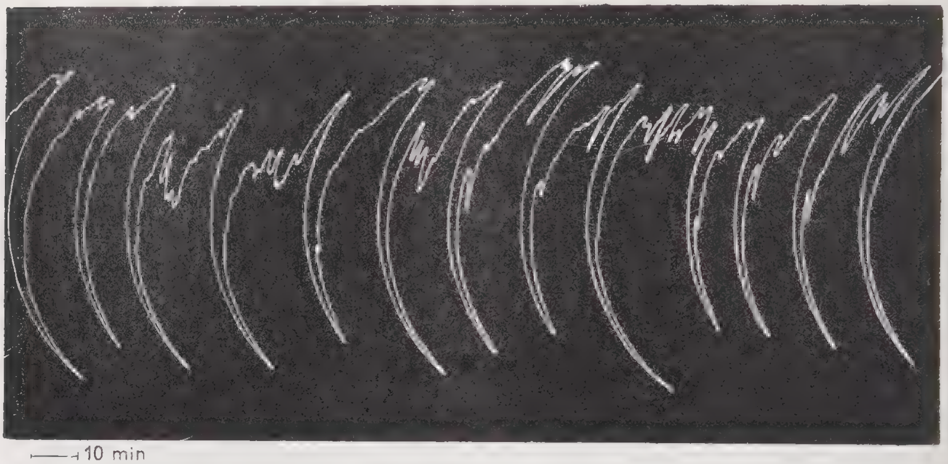

Fig. 1. Continuous record of movements of *Calliactis* during 3 h. Thread inserted in margin and attached obliquely to gimbal lever.

It is not easy to relate these cycles of activity to the closing movements which occur intermittently in unstimulated animals. The cycles recorded on the kymograph occur more frequently than visible closing movements. Moreover, the recorded movements sometimes produce a condition corresponding to partial closure, but full closure and sustained closure are almost never seen, though this may simply be due to the pull of the lever. We know also that a good many animals show visible closing movements very infrequently, yet in our experience all *Calliactis* give evidence of repeated activity cycles on the kymograph. It seems to us that the closing movements may be regarded as one form that the main movement of the repetitive cycle may take in some animals at certain times. Further data on this matter will be presented later.

The detailed picture of these low-frequency 'beats' varies greatly from one animal to another and from day to day in the same animal. There are periods, however, usually lasting several hours, when the rate is constant and then the form is remarkably constant too. Each cycle normally consists of one main series of contractions followed by a partial relaxation and then by one or more subsidiary cycles. In typical cycles, like those shown in Figs. 1–3, ten or more distinct movements may be involved. The main movement often begins, as in Fig. 2, with a small quick contraction of the sphincter, and even when this does not appear in the record, a slight twitch or bristling of the tentacles and marginal edge is often detected visually, just before the slow downward movement of the lever starts. The main components in this movement are a slow contraction of the submarginal region, and an even shortening of the column and depression of the disk, presumably by the longitudinal mesenteric and parietal muscles. In Fig. 3 the final pull on the lever is due to a slow sphincter contraction which is powerful enough to mask the relaxation of the column taking place at the same time. Most of the big movements of the cycles consist of combinations of at least two of these components but a variety of additional minor movements may be involved in various sequences.

In the subsequent relaxation, the column lengthens, presumably by the contraction of the circular muscles, but before relaxation is complete it is generally interrupted by a subsidiary cycle. This is characterized by the constriction and twisting of the column at the level of the cinclides, initiating a peristaltic wave of contraction which moves up the column and which may involve the margin.

Electrical stimuli giving a quick contraction of the sphincter can reproduce a good deal of this activity, and generally initiate a complete cycle conforming to the pattern of repetitive activity at the time. This suggests that the small sphincter contraction or tentacular twitching, so often seen just prior to the beginning of a cycle, may be a signal that the cycle is about to start, or may act as its trigger. Low-frequency stimuli, which elicit only slow contractions, do not initiate new cycles, possibly because several muscle systems which normally work independently are brought into action simultaneously so that the sequence is disrupted.

Activities of isolated preparations of Calliactis

Two kinds of preparation have been studied: (1) simple rings taken from the column at all levels; (2) preparations consisting of three loops taken from the margin, the submarginal region and the lower column at the level of the cinclides, joined together by a connecting strip of column.

The simple ring preparations showed that the circular muscle of the column is rhythmically active at all levels below the sphincter. Moreover, the timing of the 'beats' is very close to that observed in the movements of whole animals, usually with several main movements and rather more subsidiary movements occurring per hour (Fig. 4). Such rings give slow contractions to stimuli like those obtained from *Metridium* preparations (Batham & Pantin, 1954), and these contractions are similar to the main movements of the cycles

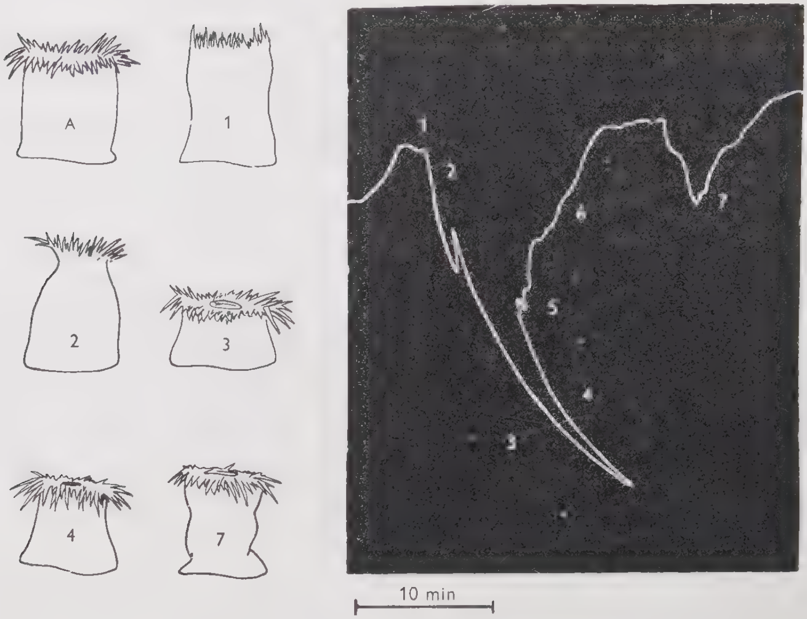

Fig. 2. Record of a single cycle in the rhythmic activity of *Calliactis* during 35 min. Numbers refer to the following distinct stages recognized visually and depicted in the accompanying sketches: (1) quick sphincter contraction; (2) submarginal contraction; (3) column shortening; (4) column extension; (5) brief submarginal contraction followed by (6) expansion of disc; (7) subsidiary cycle with transitory changes in length and peristaltic movements of the column. A shows the animal in the non-active stage.

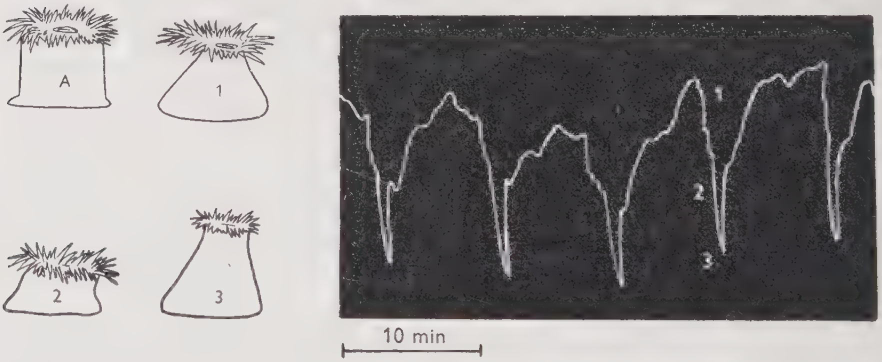

Fig. 3. Record of five cycles in the rhythmic activity of *Calliactis* during 39 min. Numbers on record and sketches refer to the following distinct stages in the movement: (1) submarginal contraction; (2) column shortening; (3) slow sphincter contraction merging into column extension. A shows the non-active stage.

(Fig. 4). As a rule ten or more stimuli are required to start such a movement and the latent periods are usually at least 1 min.

The triple-loop preparations gave evidence of co-ordinated activities. Movements occurred about every 10–20 min as in the intact animal, and in these movements, all three loops came into action in a definite sequence. Since the longitudinal strip of column joining the loops was firmly fixed to a cork plate with pins and held firmly in position by strips of glass pressed down by rubber bands, there was no possibility of the movement of one loop pulling mechanically on the next. The co-ordination seems to be based,

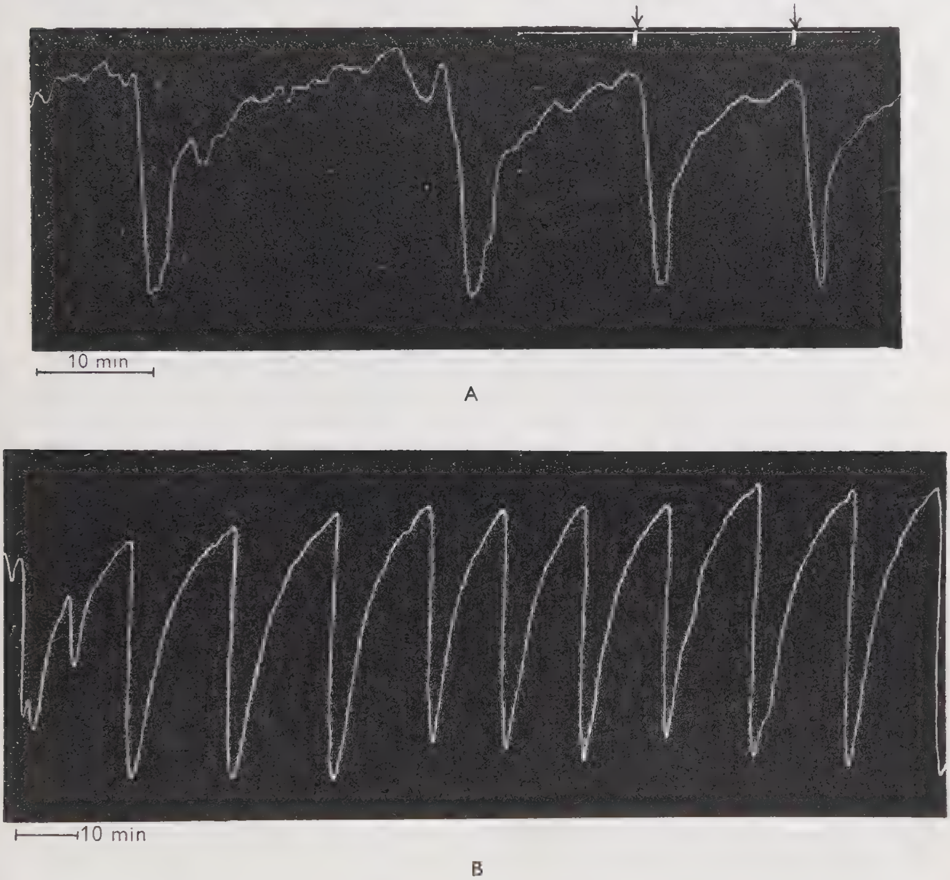

Fig. 4. Activity of simple ring preparations of *Calliactis* column. A, Preparation taken from submarginal region. 40 min record of activity without stimulation followed by period of 35 min. during which electrical stimuli (10 stimuli at 1·2 sec) were applied at 12 and 26 min (arrows). The slow delayed contractions in response to stimuli are preceded by small contractions on the stimuli probably due to residual sphincter tissue in the preparation. B, Preparation taken from lower region of column. Record of activity during 2½ h without stimulation.

therefore, on nervous, or perhaps intramuscular conduction. In the bigger movements involving all three loops, the submarginal region almost always took the lead. It began to move from 30 sec to 1 min before the sphincter and about 1 min or more before the loop from the lower part of the column. In the latter, independent movements frequently occurred. Fig. 5 shows records which illustrate these points. The general picture is similar to that found by Batham & Pantin (1954) in *Metridium*, where there is a gradient of spontaneity from the sphincter to the pedal edge, though *Calliactis* is more consistent in showing the submarginal region leading the co-ordinated movements. From the kymograph records of preparations alone, one might postulate that the pace-maker for these movements is located in the submarginal region, but we must recall that in the whole animal, a marginal twitch, often too small to be recorded, frequently precedes the main recorded

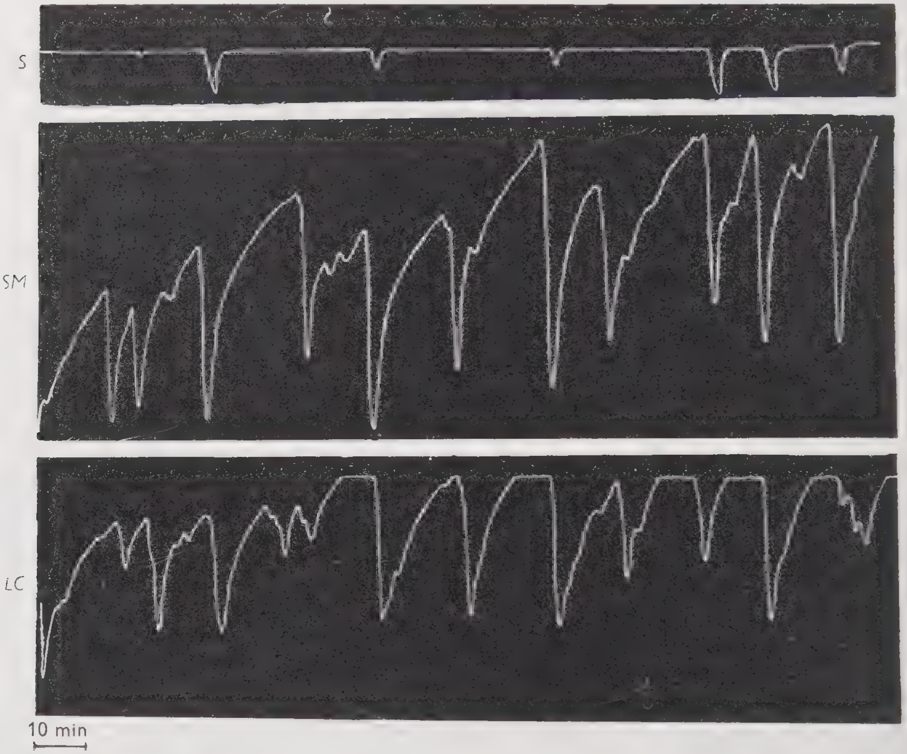

Fig. 5. Three-ring preparations of *Calliactis* with connecting strip of column recorded over 2½ h. *S*, sphincter ring attached to spring lever; *SM*, submarginal ring; and *LC*, lower column ring attached to weighted levers. The sphincter movements here recorded are slow contractions and all are linked with certain movements involving the other rings and beginning with the submarginal ring.

movement. Therefore, the location of the pacemaker must remain an open question.

The effects of stimuli on the triple-loop preparations also showed the submarginal region taking the lead in the slow responses to stimuli at frequencies of 6·1 and 7·5 sec. Even when the electrodes were nearer to the loop at the cinclide level, the submarginal loop began to contract about 30 sec before either of the other two loops.

Special experiment on five Calliactis

The results presented so far refer to an assortment of *Calliactis* studied at different times and places. In order to obtain more definite information based on a few animals studied in detail, an additional experiment was set up. Five *Calliactis* on plates were placed in a bath and visual, photographic (still) and film records of their activities were made over a period of 3–4 weeks. They were fed once weekly with chopped *Mytilus* muscle and the sea water was changed one day after feeding. After thus observing the animals under aquarium conditions, each one was removed in turn and by means of threads inserted into the marginal, submarginal and cinclide regions of the column, 3-lever kymograph records were taken to show the movements of the whole animals over periods of about 2 days. After this, 3-loop preparations were made from each animal (after 4–6 h anaesthesia in 0·4 M-$MgCl_2$) and their movements recorded on the same levers as long as the preparations survived in good condition, usually 1½–2 days.

One feature of the observations was that each animal was found to have certain individual characteristics of attitude, posture and activity which it displayed throughout the experiment. The animals are shown in Fig. 6, and they were labelled A to E from left to right. Animal A usually kept its column moderately extended and its disk depressed below the level of the margin. Its tentacles were well extended but rather limp and trailing, and from the amount of movement of the margin, the number of closures observed, and the frequency of peristalsis, it was designated 'active'. In animal B the tentacles invariably rose stiffly from the margin, the column was always elongate and erect, with the margin kept in a near-vertical position. In this animal, activity was intense for short periods, with long periods of apparent immobility in between, and it was therefore described as 'intermittently active'. *Calliactis* C showed an asymmetrical posture, with the tentacles and margin slightly lifted on one side, *Calliactis* D a squat posture, with a deeply depressed disk and very short column, and *Calliactis* E an erect posture with almost no basal expansion, and a disk tending to project above the margin. *Calliactis* C was very active from time to time and so was classed as 'intermittently active'; *Calliactis* D and E moved infrequently and showed little variation in shape and were described as 'inactive'.

Photographs *a–f* in Fig. 6, taken at 1 min intervals, illustrate the characteristic

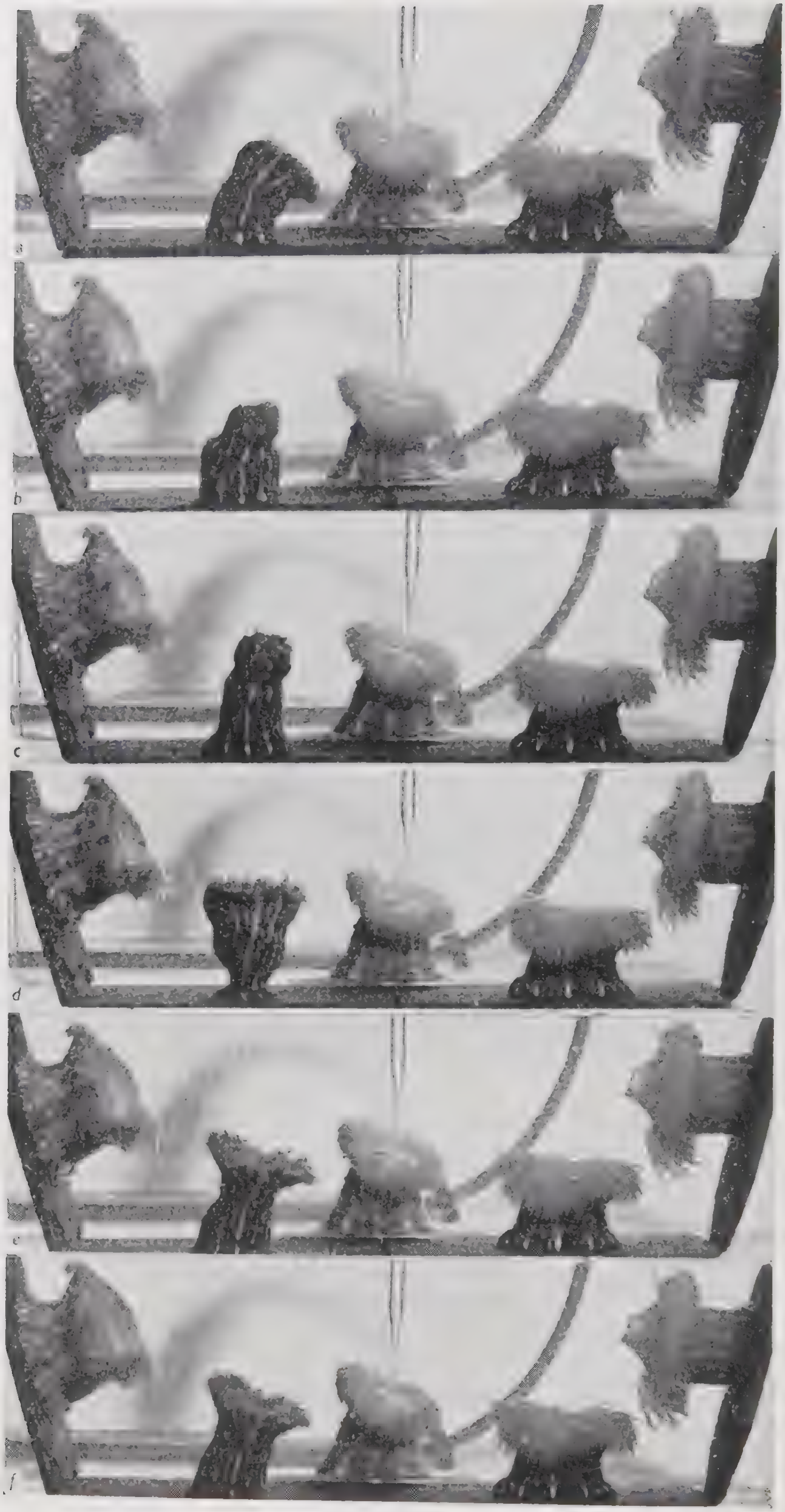

Fig. 6. For legend see opposite page.

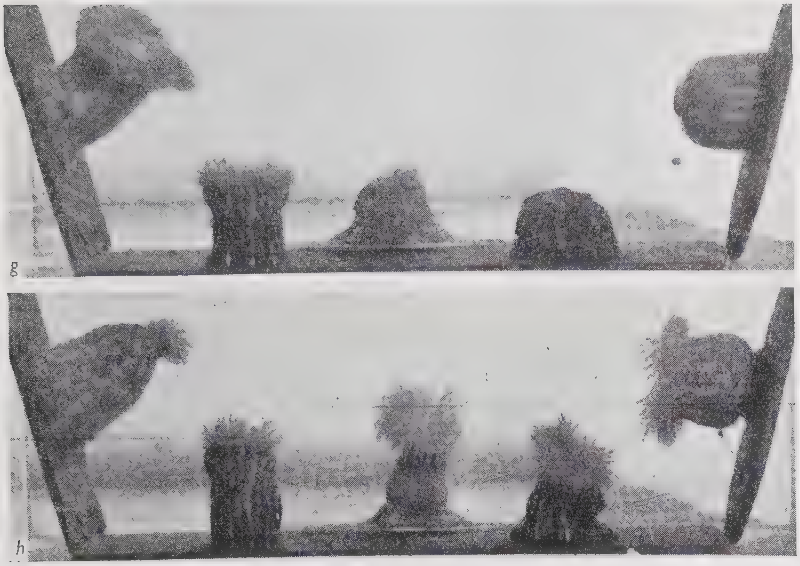

Fig. 6. Group of five *Calliactis* used in experiments described on pp. 797–803. Animals designated A, B, C, D, E from left to right. *a–f*, photos taken at intervals of 1 min showing 'normal posture' of animals A, C, D and E and active phase in animals B. *g*, 'normal posture' of animal B and active phases in animals A, C, D and E. *h*, $2\frac{1}{2}$ min after feeding.

postures of *Calliactis* A, C, D, E, and an activity cycle in *Calliactis* B corresponding to the description given earlier. The position in (*b*) was produced by a slow contraction of the margin which was maintained while the column was extended (*c*). Positions (*d*) and (*e*) marked the beginning and end of a peristaltic wave in a subsidiary cycle. The characteristic posture of *Calliactis* B is shown in (*g*). Under normal conditions, not more than 1 or 2 of these animals were active at once. Photograph (*g*), in Fig. 6 shows one instance, however, when all these animals except *Calliactis* B were either closed or in active movement, in two cases due to defaecation. Feeding, as might be expected, initiates an outburst of activity which goes on for at least 15 min. Several closing movements take place in quick succession and various distorted or asymmetrical positions are taken up before the animal returns to its normal posture and behaviour pattern. Photograph (*h*) shows the group $2\frac{1}{2}$ min after feeding. As far as could be seen, the normal rhythmic cycles were resumed within an hour or two, and were continued without marked change until defaecation initiated another outburst of activity about 2 days later.

Three-lever kymograph records of the activities of three of these *Calliactis* (A, C and E) and of 3-loop preparations made from them are shown in Fig. 7. These show the normal patterns of activity in periods outside feeding cycles lasting for 2 or 3 h. The descriptions 'active', 'intermittently active' and

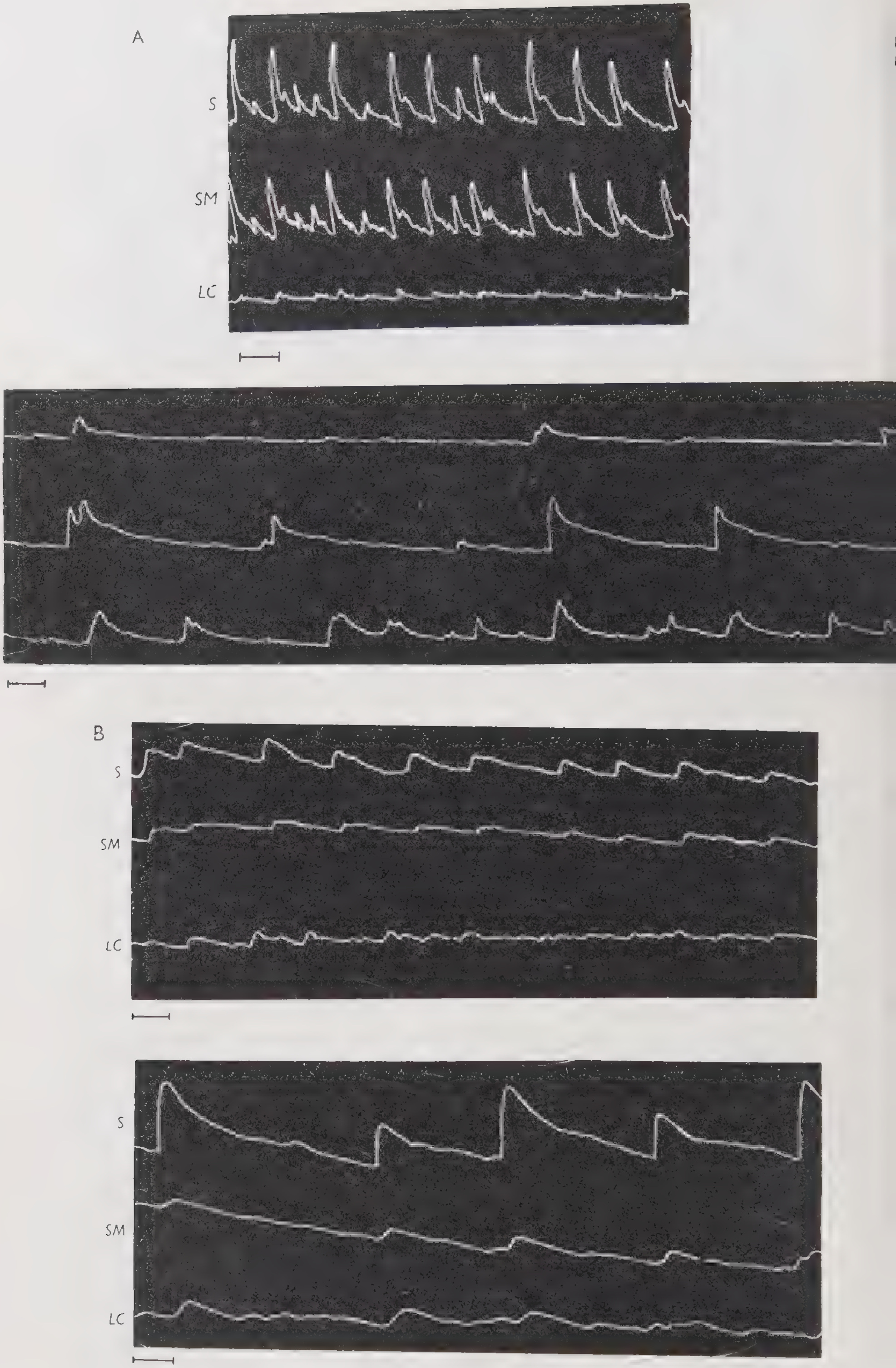

Fig. 7. For legend see opposite page.

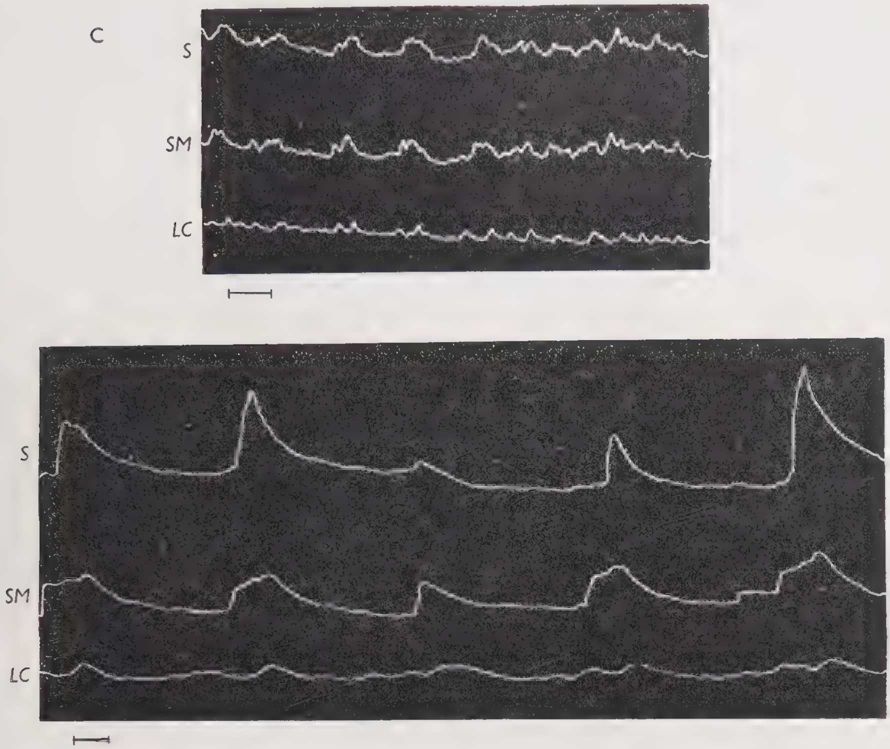

Fig. 7. Records of activity of intact *Calliactis* from the group of five experimental animals (cf. Fig. 6), with threads attached to marginal, submarginal and lower column regions, and records of three-loop preparations made later from the same animals. A, Intact *Calliactis* A (cf. Fig. 6) for 2 h and its 3-loop preparation for 4 h (7 days later); B, intact *Calliactis* C for 3 h and its preparation for 3 h (4 days later); C, intact *Calliactis* E for 2 h and its preparation for 4 h (6 days later). *S*, *SM* and *LC* indicate tracings from sphincter, submarginal and lower column regions in each recording. The scales represent a period of 10 min.

'inactive', which were applied to *Calliactis* A, C and E respectively from their behaviour over many days, are seen here to apply also to some extent to their tracings.

It is not easy to distinguish between active and passive movements at a given region in the intact animal. Big movements of the animal at any level must lead to passive movements of a lever attached to a neighbouring level, and thus the marginal and submarginal levers usually show more or less identical movements in the upper tracings in Fig. 7. In four of the five animals A–E, the submarginal region took the lead in all movements except those which began with a quick contraction, but in animal C, the regular movements began with the margin. Near the base there were many independent movements though the amplitude of these was generally much smaller than at the other levels.

Data on the number of movements in these animals, as seen in the visual, film and kymograph records, are given in Table 1. They are expressed as movements per 24 h, though for the film and visual records, these figures are obtained by multiplying up results for 6 and 20 hr respectively. It is interesting that the number of marginal closing movements observed in the visual series gives a frequency of about 1 per h which is very close to the estimate obtained in the earlier experiment. But this figure falls short of the number of movements recorded in the film and on the kymograph by a mean factor of 4 or 5, and this descrepancy applies to all the animals in this experiment.

TABLE 1. DATA ON BEHAVIOUR AND ACTIVITY OF *CALLIACTIS* A–E

Calliactis	A	B	C	D	E	
Behaviour, posture, etc.	Active, tentacles relaxed, disc depressed	Intermittently active, tentacles erect, disc depressed	Intermittently active, asymmetrical posture	Inactive, tentacles relaxed, disc depressed	Inactive, tentacles trailing, disk level or raised	Mean
No. of closures and partial closures in 24 h visual observation*	35	43	31	12	15	27
No. of marginal movements in 24 h film record†	111	204	105	27	291	148
No. of repetitive marginal movements in 24 h kymograph record of whole animal	74	177	97	211	95	131
No. of co-ordinated movements in 24 h kymograph record of 3-loop preparations	34	49	48	51	32	43

* Calculated from 20 h actual observation.
† Calculated from 6 h film record.

Difficulties in relating kymograph records to visually observed activity were encountered earlier, and it was hoped that the experiment with five *Calliactis* would clarify the matter. Instead, it has only emphasized the individual and phasic differences in the form and frequency of repetitive activity. In *Calliactis* C, D and E, the basic pattern of activity consists of movements hardly visible to the eye, appearing as slight twitches in the film and as small movements of the kymograph levers (Fig. 7 B, C). In *Calliactis* A and B, movements like those in the rhythmic cycles described above, which are associated with fairly conspicuous changes of shape or partial closure, dominate the kymograph records (Fig. 7 A), but with a frequency two or three times as great as the same movements in the visual observations. It is unlikely that such movements would have been overlooked, so it must be assumed that the kymograph records in A and B coincided in time with phases of exceptional activity.

The functions of these movements can only be surmised. However, from the changes of shape and volume accompanying the more conspicuous activity cycles, it might be inferred that some water is expelled from the coelenteron and replenished by fresh water at such times. The smaller repetitive movements of the levers seen in Fig. 7 B and C are due to small movements in the animal which lift the margin and constrict the submarginal region and other parts of the column. These movements are probably carrying out minor pumping and circulating activities, and the pattern is interrupted only at long intervals by the larger movements and closures which could provide for an exchange of water on a bigger scale.

The records of preparations made from these animals differ from those of the whole animals in many respects (Fig. 7). The frequency of the co-ordinated movements is much reduced and, although much closer to the frequency of closing movements as observed visually (Table 1), the preparations show much more regularity. Four out of the five preparations examined gave movements that were very much larger than the movements at the same level in the intact animal, though as the figure shows, the enhancement was not always the same at all levels. However, this may only mean that as preparations the loops were freer to move than in the intact column.

It is interesting that in the preparations, the individual differences noted in the intact animals tend to disappear. The timing and general form of activity in these five preparations were not very dissimilar from one animal to another and bore little relation to the record taken from the whole animal. In the co-ordinated and independent activities of the different loops, these preparations behaved like the preparations described earlier, the submarginal loops being the first to move in all except *Calliactis* C, and the loops at the level of the cinclides showing many movements of their own.

DISCUSSION AND CONCLUSIONS

The activities observed in *Calliactis* provide another example of an apparently almost immobile animal showing a great deal of co-ordinated behaviour occurring at a very slow time scale. But there is nothing simple or stereotyped about these activities. Patterns of behaviour in *Calliactis* are extremely variable with individual differences amongst animals and phasic differences in single animals at different times.

All the observations indicate that in the aquarium the quick closing response is a relatively infrequent occurrence, but it may happen more frequently when the animal is living on the shell with *Eupagurus*. Inconspicuous slow movements comprise most of the neuromuscular activity of the animal, and even a good deal of the activity of marginal sphincter itself. In the study of actinian nerve and muscle, work on the physiology of the slow activities is obviously very important. Quick facilitated contractions, notwithstanding their many interesting physiological features, are highly specialized activities in which some of the more typical and general properties of the actinian neuromuscular system may be obscured.

We feel that our observations should be regarded chiefly as another contribution to the growing literature on slow-periodic activities in invertebrate neuromuscular systems. Although the evidence from preparations suggests that these activities are co-ordinated, it is difficult to envisage appropriate mechanisms of conduction in the nerve net or intramuscular slow conduction processes to account for the exceptionally slow passage of the co-ordinated movement through the animal. But slow periodicity is a feature of many processes in which neuromuscular activity is not generally regarded as being involved. Dr L. J. Hale of Edinburgh has made a film of the growth of an *Obelia* colony by time-lapse cinematography, and the results seem remarkable to us and highly relevant to the phenomena seen in the activities of anemones. Apparently the growth of the colony is a pulsating process in which advancing movements are followed by partial withdrawals in a rhythmical way. Moreover, the timing of these 'beats' or 'pushes' is of the same order as the timing of the recorded 'beats' in both *Metridium* and *Calliactis*, occurring every few minutes (Hale, personal communication). Perhaps the notion that there is an underlying slow periodicity in the lives of certain organisms, involving their growth and development as well as their behaviour, is one that might be borne in mind in this connexion.

Slow periodic activities are now known to be involved in the behaviour of widely different groups of animals, in burrowing worms (Wells, 1950), in tunicates (Hoyle, 1952), as well as sessile coelenterates. Although these activities are generally described as spontaneous because they often have no obvious external or internal causes, and particularly because they are often seen in isolated preparations, they have many features that deserve further investigation. Koshtoyantz & Smirnova (1955) reported a relationship between the availability of sulphydryl groups and the initiation and maintenance of slow rhythmic movements in *Actinia*. We repeated their experiments on ring preparations of *Calliactis* but did not find that cadmium chloride inhibited, or that cysteine re-started, slow periodic movements in the way they had described. Yet this approach could be very useful if applied generally to this problem. Experiments aiming to reveal the properties of these spontaneous slow periodic activities by testing the action of a wide range of treatments, using metabolic as well as neuromuscular agents, might produce results throwing a great deal of light on this still obscure corner of comparative physiology.

We acknowledge with very warm thanks the help we received from the Director and Staff of the Plymouth Laboratory where much of the work was carried out. We have also had invaluable assistance from Mr W. Brackenbury, who did the photography, and from Mr Norman MacQueen of the MacQueen Film Organization, who took the film sequence. One of us (M.N.) also thanks Prof. P. B. Medawar, F.R.S., for the facilities and hospitality received at the Zoology Department, University College, London, during several months spent there in 1954–55 as a visiting research worker from Canada.

SUMMARY

Features in the behaviour of the sea anemone, *Calliactis parasitica*, as seen in visual, photographic and kymograph records, are described.

As well as occasional and aperiodic closures, sometimes maintained for several hours, this anemone shows slow, complex, co-ordinated activity cycles whose main components are repeated at fairly regular intervals. These occur several times per hour in most animals, but their form and frequency show many individual and phasic variations. Slow movements of the marginal sphincter contribute to these activities.

Contractions of the submarginal region generally lead the slow co-ordinated movements in whole animals and 3-loop preparations, but tiny marginal twitches often precede them in whole animals. Loops and ring preparations taken near the base show most independent activity.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ABBOTT, B. C., HILL, A. V. & HOWARTH, J. V., 1958. The positive and negative heat production associated with a nerve impulse. *Proc. roy. Soc.*, B, Vol. 148, pp. 149–87.

'Initial' heat production in non-medullated nerve (*Maia*) has been re-examined with improved instruments. A single impulse at 0° C gives an observed deflexion corresponding to two distinct phases of heat: (*a*) heat production of about 9×10^{-6} cal/g nerve within 20 msec of the impulse, immediately followed by (*b*) heat absorption of about 7×10^{-6} cal/g nerve lasting about 300 msec. The net heat of 2×10^{-6} cal/g nerve confirms previous estimates. Heat is contributed by many fibres of varied diameter and velocity of propagation. As a result the positive and negative phases of heat production are, to some extent, masked by the asynchronous arrival at the thermopile of impulses of different velocity. Using probable assumptions for propagation velocity the corrected positive and negative heat production becomes about 14×10^{-6} and 12×10^{-6} cal/g nerve respectively.

In the discussion some possible sources of the heat are considered. The discharge and subsequent recharge of the membrane capacity, though giving positive and negative heat of the observed magnitude have the wrong time relations to be the source of the heat recorded in the present experiments. Heat of mixing in the trans-membrane exchange of Na and K ions may contribute part of the positive heat. The heat production accompanying chemical events in the membrane during its cycle of permeability change is suggested as a likely source of heat, positive and negative.

J.V.H.

ABBOTT, B. C. & LOWY, J., 1958. Contraction in molluscan smooth muscle. *J. Physiol.*, Vol. 141, pp. 385–97.

Certain mechanical and thermal events during activity of long-fibred smooth molluscan muscles have been studied. In all cases the speed of shortening was found to be related to load during isotonic contraction in a manner approximating closely to Hill's characteristic equation. It was also observed that shortening of stimulated muscle is accompanied by extra heat production proportional to shortening. In the smooth muscles studied, the constant a for shortening heat differs from the value a in the characteristic equation.

The smooth muscles develop greater tensions, have a slower maximal shortening velocity, a smaller maintenance heat rate, and relax more slowly than any other type of muscle hitherto studied.

Following a release during the plateau of an isometric tetanus, re-development of tension decreases with speed of release. Fall of tension after stretch of tetanised muscle also decreases with speed of stretch. For a given extent of lengthening, the maintained tension increase above isometric is more pronounced than in vertebrate skeletal muscle, i.e. the active smooth muscles show a greater rigidity.

The ability of the smooth muscles to maintain high tensions economically is discussed in terms of their slow relaxation, small maintenance heat rate, and the extent of 'setting' within the contractile material as a result of activity. Tension is assumed to be maintained by spontaneous random excitation of the muscle, rather than by a special 'catch' mechanism.

J.L.

ABBOTT, B. C. & LOWY, J., 1958. Mechanical properties of *Helix* and *Mytilus* muscle. *J. Physiol.*, Vol. 141, pp. 398–407.

Experiments with smooth muscle preparations from *Helix* and *Mytilus* have shown that maximum isometric tension is developed at about the longest length reached by the muscles in the animal's body (reference length). When stretched beyond reference length, the muscles exert resting tension. This is not due to spontaneous activity.

Relaxation, following isometric contraction, and stress relaxation (decay of tension following rapid stretch of resting muscle) are not identical processes as claimed by Bozler. Their time courses differ and stress relaxation is not significantly affected by changes in temperature.

J.L.

CARLISLE, D. B., 1958. Niobium in Ascidians. *Nature, Lond.*, Vol. 181, p. 933.

Niobium has been shown to be present in some species of Ascidians. In *Molgula manhattensis* some individuals possess vanadium, others niobium. The two elements do not appear to be present in significant amounts in the same individual.

D.B.C.

CARLISLE, D. B. & HUMMERSTONE, L. G., 1958. Niobium in sea-water. *Nature, Lond.*, Vol. 181, pp. 1002–3.

There is enough niobium in inshore sea water to supply the needs of those ascidians which have been shown to contain it. The method is described for detecting small amounts of the element in sea water. The different samples vary between 0·005 and 0·1 μg/l.

D.B.C.

DENTON, E. J. & WALKER, M. A., 1958. The visual pigment of the conger eel. *Proc. roy. Soc.*, B, Vol. 148, pp. 257–69.

New methods of studying the spectral absorption of intact retinae are described. Using these methods the retina of *Conger conger* (L.) has been studied and the retinal spectral absorption curves are compared with those obtained on retinal extracts made with digitonin solution. The retina of the conger, like that of deep-sea fish, is golden in colour, its absorption curve being similar in shape to that of frog rhodopsin but with its maximum displaced about 16 mμ towards the blue end of the spectrum. The absorption curve of unbleached retinae is displaced about 4 mμ towards the red end of the spectrum from the absorption curve of unbleached retinal extract, but, when an estimated correction for possible yellow impurities in the extract is made, this displacement is only one of 2 mμ. The change in optical density of the dark-adapted retina on bleaching with strong white light is 0·6 at $\lambda = 484$ mμ: this probably represents a retinal density for unbleached pigment of about 0·8. The visual pigment in the intact retina is approximately twice as effective as simple calculations based on extracts would predict. The absorption of light by the retina is dominated by the principal photosensitive pigment, whilst the screening of the rods, due to the absorption of light by the layers of retina lying between the rods and the internal limiting membrane, is trivial.

E.J.D.

North, W. J. & Pantin, C. F. A., 1958. Sensitivity to light in the sea-anemone *Metridium senile* (L.): adaptation and action spectra. *Proc. roy. Soc.*, B, Vol. 148, pp. 385–96.

The sea-anemone *Metridium senile* responds to illumination by local contraction of the longitudinal musculature, particularly of the parietal system. No specific receptors have so far been identified for this response. The phenomena of light and of dark adaptation are shown by the photoreceptive mechanism. The action spectra of white individuals show a maximum at about 490–520 mμ. Relative to this the sensitivity in the region 550–600 mμ varies in different individuals. The possible presence of two distinct photosensitive systems is mentioned. Sensitivity decreases very rapidly beyond 600 mμ. Action spectra of the white, red and brown coloured varieties of the species are compared in relation to the absorption spectra of tissues obtained from the three types. It is concluded that the pigments which give the characteristic body colour in the red and brown varieties act as filters. Comparison with the white variety indicates that these pigments probably do not act as specific photosensitive substances. It is pointed out that the differential sensitivity of the coloured varieties appears to have no selective influence on the occurrence and distribution of the different coloured varieties.

C.F.A.P.

Robson, Elaine A., 1957. The structure and hydromechanics of the musculo-epithelium in *Metridium. Quart. J. micr. Sci.*, Vol. 98, pp. 265–78.

Musculo-epithelial cells in the mesenteries of the sea-anemone *Metridium senile* contribute both to the muscle-field above the mesogloea and to the epithelium. The epithelial elements are connected to the muscle fibres by vertical protoplasmic strands, and the intervening space is occupied by fluid. This subepithelial fluid forms a thin layer between the epithelium and muscle-field.

Epithelial elements from contracted mesenteries are taller than those from stretched tissue. As the area of the mesentery decreases during contraction, a reversible change from pavement to columnar epithelium takes place. The epithelium is able to follow rapid contractions without delay, owing to the hydrostatic action of the subepithelial fluid in thrusting it outward. Although considerably elastic, the epithelium does not appear to be active during contraction or relaxation and is moved passively.

Only the coelenterates possess true musculo-epithelium, and it may be modified, as in certain regions of *Metridium.* Analogous systems occurring in higher animals are discussed. It is possible that the subepithelial fluid of *Metridium* not only functions hydrostatically, but that it and the mesogloeal fluid together form an ‘internal medium’ providing some degree of biochemical co-ordination.

E.A.R.

Sutton, Muriel F., 1957. The feeding mechanism, functional morphology and histology of the alimentary canal of *Terebella lapidaria* L. (Polychaeta). *Proc. zool. Soc. Lond.*, Vol. 129, Part 4, pp. 487–523.

The food of *Terebella lapidaria* is collected by the mucus-secreting tentacles and passed to the upper lip and thence to the mouth. The lower lip is not concerned with food ingestion; its function is to provide mucus and cement for tube building.

The complex musculature of the lower lip has been described and separate coelomic cavities within upper and lower lip regions noted. The musculature of upper and lower lips together with the varying pressures of the coelomic fluid in upper and lower lip regions and in the thorax are responsible for lip movements.

The structure of the alimentary canal has been described in some detail, and, in particular, an account has been given of a complex type of mucous secretion involving a glandular epithelium and an adjacent glandular tissue. In no part of the alimentary canal is there a peritrophic membrane, *sensu stricto*.

The possible course of evolution of the arthropod alimentary canal from that of a polychaete ancestor is discussed. M.F.S.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1957–58

The Council have to report with regret the death of two distinguished members of the Association: Admiral Sir Aubrey C. H. Smith, K.B.E., C.B., M.V.O., who represented the Fishmongers' Company on the Council from 1937 to 1946 and was a Vice-President of the Association from 1948 until his death; and Prof. H. U. Sverdrup, Director of the Norwegian Polar Institute, who was elected an Honorary Member of the Association in 1949.

The Council and Officers

Four ordinary meetings of the Council were held during the year, two in the rooms of the Royal Society, one at Fishmongers' Hall, and one at Plymouth. At these the average attendance was sixteen. The Council wish to record their thanks to the Prime Warden and Court of the Fishmongers' Company for their hospitality in entertaining members of the Council to lunch on 23 October 1957, and making their Court Room available for the meeting on that afternoon.

It has been a source of much pleasure to the Council that our Honorary Treasurer, Mr Harrison S. Edwards, has been Prime Warden of the Fishmongers' Company during the year.

The Plymouth Laboratory

During the year the new outside circulation tanks have been completed and the new pumps installed. The circulation from the new sea-water reservoirs has now come into operation, and this supplies the research tanks on the first floor of the main laboratory as well as most of the outside tanks.

Minor alterations have been made to enlarge the room in the north building adjoining the dark-room, and it has now been fully equipped for photography.

Aquarium

During the year structural alterations were made in the old sea-water reservoirs whereby silt from the water returning from the aquarium is trapped in one of the reservoirs. From this reservoir clean water overflows into the other, from whence it is pumped back to the aquarium. As a result of this the water in the aquarium tanks is now exceptionally clear.

Owing to the breaking up of their slate walls several tanks on the south side of the aquarium have developed serious leakages. Two of them had to be emptied and put out of use during the busiest period in the summer. Temporary repairs to four tanks have been effected, but it is evident that rebuilding of the tanks cannot be much longer delayed.

Plans are in preparation for rebuilding, and Dr D. P. Wilson, in company with our architect, Mr F. L. Preston, F.R.I.B.A., of Messrs Easton and Robertson, visited the recently reconstructed aquarium of the Oceanographical Institute at Monaco in September.

Work on reconstruction of the tanks will probably begin in the autumn of 1958 and this has been made possible by generous grants from the Nuffield Foundation and other sources which are recorded on page 23 of this report.

RESEARCH SHIPS

The three research ships have operated regularly throughout the year. R.V. 'Sarsia' has satisfactorily completed her first four years' continuous machinery survey and special survey required by Lloyd's Register of Shipping. R.V. 'Sula' has also satisfactorily completed her second four-year continuous machinery survey. During the year a new rudder stock was fitted, and a heat exchanger has been fitted enabling the engine to be cooled by fresh water.

M.V. 'Gammarus' has been running continuously, but a thorough inspection of her hull will soon be necessary.

RAY LANKESTER INVESTIGATOR

The Council have pleasure in reporting that Prof. J. E. Harris, F.R.S., has been appointed Ray Lankester Investigator to work at the Plymouth laboratory in 1958.

STAFF

Dr H. W. Harvey, C.B.E., F.R.S., retired from the staff of the Plymouth laboratory on 31 March 1958. The Council wish to record their deep appreciation of Dr Harvey's services to the Association over a period of nearly 37 years. His distinguished researches on the productivity of the sea and the physiology of diatoms have added much to the international reputation of the Plymouth laboratory. The good wishes of the Council go with him on his retirement. Dr Harvey was appointed Commander of the Order of the British Empire in the New Year Honours for 1958.

Miss D. Ballantine resigned from the staff of the Plymouth laboratory in January 1958 on her marriage.

Dr E. D. S. Corner joined the staff of the Plymouth laboratory as Senior Scientific Officer on 1 September 1957. He has been seconded to the International Paints Research Fellowship for the year ending 31 August 1958.

Dr B. C. Abbott left in August 1957 on special leave of absence to work in the University of California at Los Angeles.

Mr B. R. Jewell has been appointed temporary Scientific Officer on the staff of the Plymouth laboratory during Dr Abbott's absence.

Dr L. H. N. Cooper attended meetings of the International Union of Geodesy and Geophysics in Toronto and of the International Council for the Exploration of the Sea in Bergen, in September and October 1957.

Dr D. B. Carlisle attended the second International Symposium on Neurosecretion in Lund in July 1957. He also visited the Kristineberg Marine Laboratory and the Max-Planck Institute for Biochemistry in Munich during the year.

Mr F. A. J. Armstrong attended the meeting of the International Council for the Exploration of the Sea held in Bergen in September and October 1957.

Dr T. I. Shaw attended a Colloquium on the Ecology of Marine Algae held at Dinard in September 1957 under the auspices of the Centre National de la Recherche Scientifique.

Occupation of Tables

The following one hundred and forty-eight workers have occupied tables at the Plymouth laboratory during the year:

E. Adams, Plymouth (Library).
V. P. Agrawal, London (Mouthparts and feeding of amphipods).
R. McN. Alexander, Cambridge (Swim bladder of teleosts).
Dr J. S. Alexandrowicz, Plymouth (Crustacean nervous system).
T. D. Allan, Cambridge (Geology of continental shelf).
Dr Daphne Atkins, Plymouth (Ciliary mechanisms of brachiopods).
T. B. Bagenal, Millport (Fecundity of plaice).
R. Bassindale, Bristol (Barnacle morphology).
Dr J. S. Bradshaw, La Jolla (Foraminifera).
Dr Anna M. Bidder, Cambridge (Digestive system in cephalopods).
E. J. Binyon, London (Tonic regulation in *Asterias*).
Dr P. E. Brockman, Plymouth (Library).
Miss S. M. L. Bocks, London (General).
Q. Bone, Oxford (Feeding mechanisms and neurosecretion in *Amphioxus*).
A. D. Boney, Plymouth (Ecology of red algae).
Dr Eleanor M. Brown, London (Parasitic protozoa).
Sir Edward Bullard, F.R.S., Cambridge (Geology of continental shelf).
Dr C. Burdon-Jones, Anglesey (Gastropods).
Miss E. A. M. Burley, Leicester (Library).
R. F. Burton, London (General).
Mrs P. A. Caldwell, Plymouth (Library).
Dr P. C. Caldwell, Beit Memorial Fellow; Alan Johnston, Lawrence and Moseley Research Fellow (Muscle and nerve physiology).
G. W. Cambridge, Leeds (Pharmacology of lamellibranch muscle).
L. Carter, Brixham (Library).

P. E. Cloud, Jr., Washington (Oceanography).
J. W. Coles, London (Nematodes parasitic on sea-weeds).
D. J. Collins, Plymouth (Library).
R. G. Collins, Penzance (Library).
Dr E. D. S. Corner, International Paints Research Fellow (Effects of toxic substances on marine organisms).
Miss W. A. M. Courtney, London (Respiration and feeding of cirratulids).
W. D. G. Cox, Plymouth (Library).
Dr D. J. Crisp, Anglesey (Ship design).
C. J. Croft, Plymouth (Library).
Dr R. I. T. Cromartie, Cambridge (Quinone pigments of *Antedon*).
R. I. Currie, N.I.O. Wormley (Productivity of the sea).
K. W. Daisley, Shinfield (Vitamin B_{12} in sea water).
Dr R. Phillips Dales, London (Metabolism of polychaetes).
P. M. David, Godalming (Oceanic squid).
B. N. Desai, Anglesey (Gastropods).
P. S. B. Digby, London (Tissue culture; plankton).
Miss E. J. Dimelow, New Brunswick (Physiology of *Antedon*).
Dr D. T. Donovan, Bristol (Submarine geology).
Capt. J. T. Dunbar, Kingsbridge (Library).
Dr E. Elkan, Watford (Pituitary and saccus vasculosus of fishes).
Dr R. Endean, Brisbane (Vanadium and iron in ascidians).
D. Etherington, London (*Phascolion*).
Mrs D. Etherington, London (*Ectocarpus*).
Surg.-Lt. R. J. Fallon, R.N., Plymouth (Library).
Dr L. R. Fisher, Reading (Vitamin A in zooplankton).
L. H. Flavill, Cambridge (Geology of continental shelf).
R. F. H. Freeman, London (Respiration of *Scrobicularia*).
Dr Vera Fretter, Reading (Prosobranchs).
Dr T. Gascoigne, London (*Pelta*).
Dr J. B. Gilpin-Brown, Yelverton (*Nereis*; experimental light trapping).
E. Gottlieb, Haifa (General).
Prof. A. Graham, Reading (Prosobranchs).
Dr J. Green, London (Parasites of crustacea).
D. N. F. Hall, Colonial Office (Penaeid prawns).
R. Hamond, Holt (Library).
Dr J. P. Harding, London (Harpacticid nauplii).
M. G. Hardy, Reading (Rhythmical activity in bivalves).
Prof. J. E. Harris, F.R.S., Bristol (Vertical migration of copepods).
D. Heddle, Oxford (Behaviour and histology of asteroids).
Dr R. H. Hedley, London (Foraminifera).
B. T. Hepper, Conway (Lobster marking).
E. F. Hermann, Copenhagen (Physical oceanography).
Prof. A. V. Hill, F.R.S., London (Heat production in nerve).
Dr M. N. Hill, Cambridge (Structure of the continental Shelf).
M. Hornsey, Cambridge (General).
R. V. Howarth, London (Heat production in nerve).
Dr D. I. D. Howie, Dublin (Culture of *Bucephalopsis*).
Dr G. M. Hughes, Cambridge (Respiratory movements of teleosts).
D. J. Hume, Teignmouth (Library).
O. D. Hunt, Newton Ferrers (Library).

Dr C. H. Jellard, Plymouth (Library).
Dr Janet Jellard, Plymouth (Library).
Miss P. M. Jenkin, Bristol (Library).
Miss J. M. Johnson, London (General).
Dr Joanna M. Kain, Port Erin (Isolation of *Prorocentrum*).
P. J. Keady, Galway (Plankton; Library).
Dr G. Y. Kennedy, Sheffield (Porphyrins and chlorophyll).
Dr R. D. Keynes, Cambridge (Nerve physiology of squid).
M. C. Kingwell, South Brent (Library).
Prof. L. H. Kleinholz, Oregon (Crustacean endocrinology).
Sir Francis G. W. Knowles, Bart, Marlborough (Crustacean endocrinology).
Miss A. Kuku, London (General).
Dr Marie V. Lebour, Plymouth (Decapod crustaceans).
Dr J. Llewellyn, Birmingham (Trematode parasites of fishes).
Dr G. A. C. Lynch, Plymouth (Library).
Prof. Irene Manton, Leeds (Marine flagellates).
Dr Sheina M. Marshall, Millport (Respiration of *Calanus*).
A. L. Martin, London (Feeding and digestion in gammarids).
D. H. Matthews, Cambridge (Geology of continental shelf).
R. B. Mayne, Plymouth (Library).
Prof. J. L. Mohr, Los Angeles (Protozoan parasites of crustacea; Mesozoa).
Miss F. M. E. Molloy, Harpenden (Digestion in mysids).
Mrs B. Beecher Moore, London (Digestive glands of *Idotea*)
Dr J. E. Morton, London (Mollusca).
L. A. Mound, London (General).
Dr Margaret Naylor, Hull (Cytology of *Phaeophyta*).
Prof. Lily Newton, Aberystwyth (Algae).
Dr C. I. O. Olaniyan, Nigeria (Library).
Mrs E. Oldfield, Guildford (Embryology of *Lasaea*).
Dr A. P. Orr, Millport (Respiration of *Calanus*).
Prof. F. Pautsch, Gdynia (Chromatophores of crab zoeas).
C. R. Pearson, Brixham (Library).
Miss C. S. Pearson, Oxford (Brains of fish).
Dr R. B. Pike, Millport (Decapod larvae).
Dr W. T. W. Potts, Birmingham (Adductor muscle of *Pecten*).
Cdr. C. F. B. Powell, R.N. (Rtd), Plymouth (Library).
J. D. Pye, London (Nervous chromactivation in teleosts).
A. M. Qureshy, London (Blood parasites of fishes).
Miss P. M. Ralph, Wellington, N.Z. (Hydroids and medusae).
Dr F. A. Richards, Woods Hole (Chemical oceanography).
Dr F. H. Rigler, Toronto (Ion exchange in *Bryopsis*).
Prof. P. Roa Morales, Venezuela (Chemistry of sea water).
M. B. V. Roberts, Cambridge (*Myxicola* muscle).
A. H. W. Robinson, Leicester (Shallow water morphology in Start Bay)
G. Rodriguez, Venezuela (Ecology of sandy shores).
Prof. Findlay E. Russell, Los Angeles (Toxins of weevers).
M. Saleem, Manchester (Crustacean mouth parts).
Miss R. S. M. Savage, London (General).
R. J. Skaer, Cambridge (General).
Dr Eve C. Southward, Plymouth (Polychaetes).
Dr S. Smith, Cambridge (Library).

B. W. Sparrow, Newton Ferrers (Library).
Miss F. A. Stanbury, Plymouth (*Cladophora*).
O. Suddaby, Plymouth (Library).
Dr Muriel F. Sutton, London (Embryology of salps).
Dr T. K. Szela, Kettering (Library).
H. Thiel, Hamburg (General).
G. E. Walster, Plymouth (Glycolysis in *Maia*).
Dr B. Werner, List a Sÿlt (Hydroids and medusae).
Dr Mary Whitear, London (Proprioreceptors in crustaceans).
Prof. W. F. Whittard, F.R.S., Bristol (Submarine geology).
J. H. Wickstead, Colonial Office (Plankton).
Dr J. J. Wolken, Pittsburg (Photoreception).
M. Yoshida, London (Naphthaquinone pigment in *Psammechinus*).

Among the many other scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Dr G. J. Schuringa (Holland), P. de Wolf (Holland), A. M. Christensen (Copenhagen), Prof. K. Sembrat (Poland), Dr D. Goldmann (U.S.A.), S. Nagappa (India), Prof. Dr Gunnar Thorson (Copenhagen), Prof. F. E. Eggleton (U.S.A.), Dr J. Verwey (Holland), Dr P. Korringa (Holland), Dr Windemuller (Holland), Dr H. Holtedahl (Bergen), Prof. S. F. Bush (Natal), Dr P. Radoman (Yugoslavia), Prof. R. A. Jasper (Canada), Dr P. Gurtner (Rome), Mrs N. Tirmizi (Pakistan), Miss M. Kalk (Johannesburg), Dr E. Lisitzin (Finland), Dr F. D. Ommanney (Hong Kong), Dr Irving Friedman (Washington), Dr V. Worthington (U.S.A.), F. P. Mendes (Lisbon), Prof. L. R. Richardson (Wellington), Dr A. G. Wurtz (France), Mr and Mrs Foster (U.S.A.), Mrs E. Finch (Australia), V. Krishnamurthy (India), Dr Katherine Tansley (U.S.A.), S. J. Holt (Rome), J. W. Brodie (New Zealand), Dr Talbot H. Waterman (U.S.A.), W. G. Morison (Sarawak), J. L. Reid, Jr. (U.S.A.), Prof. J. Tokida (Japan), Dr R. W. Hiatt (Hawaii), Dr M. S. Gordon (U.S.A.).

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty students from the following Universities: Oxford, Cambridge, Glasgow, Aberdeen, London, Liverpool, Sheffield, Reading, Southampton, Exeter, University College of North Wales, Aberystwyth and Regent Street Polytechnic.

Also during the Easter Vacation Mr J. F. Eggleton and Miss J. Kenworthy brought a party of thirteen from Hinckley Grammar School; Mr K. W. Wilkes brought four boys from Harrow School; Mr G. Thomas brought three boys from Ilford County High School, and Dr Margaret Christianson a party of nine girls from Harrow County Girls School.

Prof. J. E. Smith, F.R.S., Dr. G. E. Newell and Dr J. E. Morton conducted a course in marine biology in September for twenty-two students, mainly from Queen Mary College. Also during September Dr Margaret Naylor, of the University of Hull, conducted a course in marine botany for fifteen students.

SCIENTIFIC WORK OF THE PLYMOUTH LABORATORY STAFF

Sea Water and Plankton

Dr L. H. N. Cooper has continued his study of the effect of deep oceanic circulation upon the biological productivity of shallow seas. He has prepared four papers by invitation. One was for 'Travaux du Centre de Recherches et d'Etudes Océanographiques' on 'Experimental Oceanography in the Mediterranean'. He has suggested that the sea area off the Italian Riviera, including the Capraia Channel, may be used as a small-scale model for studies on the formation of North Atlantic deep water. Another on radioactive tracers as tools for the oceanographer was delivered before the Geophysical Section of the Royal Astronomical Society. The third under the title 'The effect of continental slopes upon vertical and horizontal circulation and upon energy transfer at depth in the North Atlantic Ocean' was written to be presented at the Toronto meeting of the International Association of Physical Oceanography. It was then found that at least a part of the variations in oxygen upon which conclusions hung was due to absorption by corrosion products in the reversing bottles. Though the conclusions are probably sound, publication must be postponed pending repetition of the work with bottles which are above suspicion. This experience was reported in lieu of the paper planned. It was possible also to report that within three weeks in June 1956 all the water in the north-eastern Bay of Biscay between 300 and 3800 m was uplifted bodily by 75 m. The cause is not understood.

At the symposium held at Bergen under the auspices of the International Council for the Exploration of the Sea on Measurement of Primary Production in the Sea Dr Cooper reported on the measurement of production by means of the consumption of nutrients. This method, once so powerful, is not easily adapted to assess the fine structure of events which we now need to know. In the English Channel it has served its turn and other means of attack need now to be sought.

At the Toronto meeting Dr Cooper was appointed chairman of a committee of the International Association for Physical Oceanography, on Chemical Oceanography, one of whose assignments is to consider standardizing methods for the analysis of sea water. He favours the devising of standards of performance with which methods must comply. Standards of this kind do not hinder advance whilst inspiring confidence in results. It remains to be seen whether the necessary intercomparison of methods between laboratories is feasible.

Dr Cooper and Dr T. I. Shaw have carried out a theoretical study on the state of iodine in sea water. The oxidized form of the element, which various authors have shown to co-exist with iodide in the sea, is probably hypoiodous acid. This hypothesis accounts for a wide range of observations, such as the ratio of oxidized to reduced forms, the iodine content of the atmosphere and the distribution of iodine between partially enclosed sea areas. The work should prove of value in predicting the oxidation states of other marine materials which undergo ready reactions with iodide and iodine.

Mr F. A. J. Armstrong has continued the monthly cruises to Station E 1, and analyses for phosphate, total phosphorus and silicate up to the end of 1956. Since then this work has been done by Mr E. I. Butler. In 1956 the winter maximum phosphate concentration was 0·51 μg atom P/l., a normal value in recent years, and markedly lower than the exceptional high value in 1955. The station was worked four times in four weeks in March and April, when there was a period of bright settled weather; changes in nutrient concentrations were observed during the spring outburst of plankton. There is good reason to suppose that the water mass at the station did not change during this period. Consumption of phosphate was first rapid and then slowed down. Silicate fell sharply and linearly during the first three weeks, to exceptionally low values (0·08–0·09 μg atom Si/l. even below the photosynthetic zone). This requires explanation, since at the time there was a thermocline and vertical mixing should not have had much effect in impoverishing the whole water column. Moreover, phosphate was not unusually low in the deeper water. During these three weeks, the ratio Si:P consumed was about 10:1 by weight. If the phytoplankton were mainly diatoms, this would suggest that they were deficient in silicon, and it is possible, since they sometimes sink rapidly in spring, that they continued, at least for a while, to remove silicon from the deeper water, even if unable to make normal growth. A week later silicate in the deeper water had increased markedly. These results are being prepared for publication. In 1957 Mr Armstrong has done analyses for ammonia by distillation followed by the indophenol blue method, and for total inorganic nitrogen by determination of ammonia after reduction with Raney nickel. The results at Station E 1 show maximum inorganic nitrogen concentrations of about 10 μg atom N/l., with ammonia about 3 μg atom N/l. Minimum values were about one-tenth of these. The results resemble those got by Dr Cooper in 1933. Simpler methods for ammonia and nitrate are being sought, and some success has been achieved in the reduction of nitrate to nitrite under ultra-violet irradiation. In distilled water, 80% reduction has been attained in the presence of urea, with manganese as catalyst, but in sea water recovery is only about 50%. Further work on this and on changes in nitrogen contents of sea water during storage is in hand.

The method and results of analysis for iron content of sea water have been published in Vol. 36, No. 3, of the *Journal*. The final model of the filter absorptiometer has been made and tried out at sea. An account of it is in preparation.

Dr E. D. S. Corner, in collaboration with Dr F. H. Rigler of Toronto

University, has made a study of the behaviour of mercury in sea water. Experiments with ^{203}Hg-labelled mercuric chloride have shown that stored sea-water solutions of this compound lose mercury at a fairly rapid rate which is significantly increased when nutrients favouring the growth of bacteria are added to the sea water, and greatly diminished when the sea water is treated with various bactericides. The conditions under which bacteria develop in solution also influence the loss of mercury qualitatively. For whereas most of the mercury lost from plain sea water is in the form of a product which volatilizes from solution, a large amount of the mercury lost from sea water containing added nutrients is taken up by the walls of the glass storage vessel. An account of this work has been published in Vol. 36, No. 3, of the *Journal.*

Dr H. W. Harvey has continued experiments on rate of growth of *Phaeodactylum tricornutum* under different environmental conditions, with the aim of indicating factors, other than those already recognized, which may affect the growth rate of phytoplankton in nature. Experiments in which *Phaeodactylum* was grown in alternating periods of 12 h light and 12 h darkness showed a greater rate of carbon assimilation during illumination than when grown in continuous light. The increase was more than was accounted for by the increase in chlorophyll during darkness, and indicated that there was also a build-up during the dark periods of the substance, or its substrates, which actuates the Blackman dark reaction. This indication, based on crude experiment, was found to conform with the conclusion arrived at by Tamiya in Japan from extensive experiments with a freshwater *Chlorella.*

In cultures of *Phaeodactylum* a marked physiological change takes place in the cells when the pH rises above 8·8, resulting in much slower growth rate and the accumulation of fatty material in the cells. Experiment indicated that this was in part due to reduced availablity of iron in the medium. This marine alga, notably unexacting in its requirements for growth, was trained to grow in 1% sea water diluted with 0·024 N sodium bicarbonate; it raised the pH of this medium to 11·5 before growth ceased. An account of these exploratory experiments has been circulated to colleagues in other laboratories who are engaged upon allied problems.

A second edition of Dr Harvey's book, *Chemistry and Fertility of Sea Waters*, containing a summary of advances made since the book was written in 1954, has been published by the Cambridge University Press.

Dr Mary W. Parke has continued her study of the form range in the genus *Chrysochromulina* (Chrysophyceae), and a further paper, in collaboration with Prof. Irene Manton and Mr B. I. Clarke of Leeds University, was published in Vol. 37, No. 1 of the *Journal.* In this paper a description is given of the anatomy of the haptonema which shows that its structure differs from that of a flagellum in having seven peripheral strands and none in the centre, as compared with nine peripheral and two central in a flagellum.

Throughout the year monthly samples of sea water have been taken at E 1, at the same time as the hydrographic samples were taken. From these samples the seasonal distribution of the already described nanoplankton forms, and of those known only by a serial number in her collection, has been recorded. Several new forms were observed and some of these have been isolated in unialgal culture. The samples have also been used for testing out methods for quantitative and qualitative estimations of these small forms as a preliminary to doing routine monthly counts. With the co-operation of Mr E. I. Butler the number of samples taken for examination has now been increased and the first series taken from L 2–L 6 and E 1 (surface to 73 m) in September has already been examined and cultured, and interesting information on the range of distribution of some species has been obtained.

Under the supervision of Dr Parke, Miss I. Adams has maintained the collection of unialgal cultures of marine phytoplankton organisms throughout the year. She has also isolated a considerable number of new forms, belonging mainly to the classes Chrysophyceae and Dinophyceae, for the use of Dr Parke and Miss Ballantine. From January to the end of November, 160 cultures had been distributed for research purposes to institutions in this country and abroad, in addition to well over 100 l. of cultures of different organisms that had been grown for use by scientists working in the laboratory.

Dr S. T. Cowan, director of the National Collection of Type Cultures of the Medical Research Council, very kindly offered to try out the freeze-dry method on four cultures, representing four different algal classes, to test whether or not these marine organisms would survive this treatment. Unfortunately, however, it was unsuccessful for the organisms tested.

A paper, in collaboration with Miss D. Ballantine, on a dinoflagellate, *Exuviaella mariae-lebouriae*, n.sp., which has been maintained in culture at Plymouth since 1949 has been published in Vol. 36, No. 3, of the *Journal*.

Through the kindness of the Director, Dr D. J. Crisp, it was possible for Miss D. Ballantine to work at the Marine Biology Station, Menai Bridge, for two months, in collaboration with Dr C. P. Spencer, on the purification of extracts of the toxin from *Gymnodinium veneficum*. The preliminary extraction had been done previously at Plymouth by Dr B. C. Abbott and Miss Ballantine. Some degree of purification was attained, and the properties of the toxin indicate that it is probably a saponin. Further work on this has been abandoned for the present due to the variable toxicity of the cultures. A variety of substances (vitamins, amino-acids, etc.) have been added to cultures in an attempt to increase the growth rate of the organism and/or the toxicity, but no success has been obtained, and the experiments have been discontinued.

Miss Ballantine is now testing for toxicity to fish all the cultures in the Plymouth Collection. As yet only *G. veneficum* and *Prymnesium parvum* have been found to be toxic. She is also continuing work on the marine dinoflagellates, both on the collection of records from observation of water samples, and on systematic and taxonomic problems in some genera.

Dr F. S. Russell, in continuation of his observations on medusae collected from deep water, has described a new species of scyphomedusa, *Atolla vanhöffeni*. He has also described a new hydromedusa, *Krampella dubia*, whose systematic position is not yet clear. This medusa has the peculiar strands of tissue running through the mesogloea from the radial canals to the exumbrella surface also seen in *Amphinema krampi*. Such strands have never been described in any other medusae. The above descriptions have been published in Vol. 36, Nos. 2 and 3, of the *Journal*. Dr Russell is now beginning to collect the necessary data for the preparation of a monograph on British Scyphomedusae.

Mr G. M. Spooner has continued examination of Amphipoda from the deep-water stations worked by R.V. *Sarsia*. These are mostly of the suborder Hyperiidea, a group widely spread in plankton and in the deep water of the oceans, but virtually unrepresented amongst the littoral or benthic fauna. The scattered literature of the group has been indexed, and, as sorting and identification of samples proceeds, a synopsis is being made of the chief descriptions and illustrations of individual species, and a simplified working key for the identification of the known world species is being compiled. Existing literature is still predominantly taxonomic or distributional. Other biological data are normally hard to obtain, for instance, on specialized feeding habits and on the peculiar commensal relationships between hyperiids and medusae or salps. The new facilities for working at sea may afford chances for advancing this knowledge.

Mr P. G. Corbin has continued his examination of the regular stramin ring trawl collections. No change in the low level of macroplankton production associated with the prevailing *Sagitta setosa* conditions has been observed in 1957.

Dr. A. J. Southward has made some preliminary observations on the distribution of plankton animals in the western Channel. It is hoped that new sampling techniques will allow rapid surveys to be made of the chief indicator species. Earlier attempts to use cirripede larvae for this purpose will be continued.

Dr D. P. Wilson has been able to amplify the account of the biology of *Ianthina janthina* (L.) which was published in Vol. 35, No. 2, of the *Journal*. A large shoal of young specimens of this species stranded in the Scilly Islands in March and April 1957 and nearly 200 perfect specimens less than half an inch high were obtained. Measurements of these small shells fully confirmed the conclusion previously reached, based mainly on large shells, concerning the change in shape of the shell as it ages. A note has been published in Vol. 37, No. 1, of the *Journal*.

Dr Wilson and Mr Armstrong have completed an account, in Vol. 37, No. 1, of the *Journal*, of the work they did in 1954 and 1955 on biological differences between sea waters. Whilst several new facts emerged, these do not appear to lead any nearer to the discovery of the main cause of the differences. The most interesting observation was that sea waters filtered at the time of collection, and proved bacteriologically sterile until use, were very little different as a medium for rearing *Echinus esculentus* larvae than were the same sea waters collected at the same time in the ordinary way and not sterile. These observations, whilst incomplete, give no support to the view that bacteria present in the water at the time of collection, and multiplying in it before use, make that water unsuitable for larvae subsequently reared in it.

Macro-Flora and Fauna

'Corrections and Additions II, 1956' to 'A Preliminary Check-List of British Marine Algae' (1953, Vol. 32, of the *Journal*) has been compiled by Dr Parke and published in *Phycological Bulletin*, No. 5, pp. 36–7, May 1957.

Additions have been made during the year to the herbarium of marine algae by all members of the botanical section to try to fill the gaps in the collection, and for this purpose visits have been made to the shores of Cornwall and the Isles of Scilly.

The final stages of editing the third edition of the *Plymouth Marine Fauna* continued to occupy much of Dr Wilson's time until its publication in June 1957. Final proof correcting was very tedious and involved checking very many references. The new volume is larger than its predecessor and lists many more species.

Much of Mr Spooner's time has also been taken up in the production of the new fauna list. Accumulated records of the burrowing fauna from the collecting grounds in the Salcombe Estuary have been collated by him, and these results have been expressed in a summarized form in the introduction to this new edition.

Mr Corbin has continued to collect data on the Lucernarians, certain of the smaller fishes and the hermit-crabs. The new *Lucernariopsis* sp. found at Wembury in 1951 has for some time presented a puzzle in regard to its distribution. In a very small area on Church Reef it has always been quite plentiful and at any one visit throughout the year some two dozen or fifty or more specimens can easily be collected without stripping the locality. Elsewhere in the south-west Mr Corbin could not find it, nor did it occur in collections examined from other parts of the British Isles. In September this year, however, material from the Scilly Isles collected by Prof. L. A. Harvey of Exeter University contained two specimens among larger numbers of *Haliclystus* and *L. campanulata*. This Scilly record thus establishes the distribution of the species over an area and that it is not limited, as formerly appeared to be so, to a single point site. The species is not a recent immigrant: the British Museum collection contains specimens from Plymouth collected before 1929.

Although absent for much of the year due to illness, Mr N. A. Holme has continued working up bottom samples obtained in previous years off southern Brittany and the south-west coast of Ireland. Some observations have been made on the mode of life of members of the Tellinidae which indicate that these lamellibranchs lie buried in the sand in a horizontal position, with the left valve down. This indicates the possibility of horizontal migrations through the sand, as was suggested by earlier work on the spacing of individuals of *Tellina tenuis* on a sandy beach. Mr Holme has translated from the French, and adapted for British coasts, the book *La vie étrange des rivages marins* by E. le Danois, and this has now been published under the title *Marine Life in Coastal Waters* (*Western Europe*).

Mr G. R. Forster has continued work on sponges collected by diving. A collection is slowly being built up of specimens of the genus *Haliclona*, with the aim of providing more satisfactory descriptions of some of its numerous species, of which very few are well established. Spicule measurements are being taken to find out the range of variation within a species, which may well be greater than many of the small differences in spicule dimensions on which new species have been erected.

Two new sponge records for the Plymouth area have been made: *Crella inflata* (Bowerbank) twice taken from the Mewstone dredging ground, and an unusual bright red sponge, tentatively identified as *Raspaciona aculeata* (Johnston), which was found on a gulley wall at 14 fathoms off Stoke Point.

Many sponges were collected from the neighbourhood of Santander during the course of five days diving in June. These dives were made from the launch of the Laboratorio Oceanographico thanks to the co-operation of the Director, Dr J. Cuesta, and the Ministerio de la Marina.

Some observations have been made on the browsing of *Echinus esculentus* in aquarium tanks. These animals will feed on a variety of encrusting organisms, including some sponges, but they do not touch *Corynactis*. One *Echinus* on which a close watch was kept was seen to move away whenever it came into contact with a stone covered with *Corynactis*. This apparent distastefulness of *Corynactis* to *Echinus* may account for the general abundance of the former at depths of 10–15 fathoms where *Echinus* is also very common.

During the summer more diving has been carried out by Mr Forster in the vicinity of Plymouth; several rare species have been found. *Phoronis hippocrepia* colonizing considerable areas of *Pomatoceros* tubes have been discovered in a gulley near Penlee Point. In the same gulley and also near Revelstoke Point several *Balanophyllia regia*, Gosse's gold cup-coral, have been observed. A few specimens of *Lima* spp., burrowing lamellibranchs, have been taken by turning over boulders.

With the Robot camera some underwater pictures of sponges and various sessile animals have been taken with the aim of showing how much more expanded many of these animals are in their natural habitat than when they are living in aquaria. A waterproofed Minifon recorder has also been tested which it is hoped will greatly facilitate making quantitative estimates or counts of the rock fauna over a fairly large area.

Several rock samples from outlying submerged reefs have been obtained by diving by Mr Forster for the Department of Geology of the University of Bristol.

Dr A. J. Southward has continued his studies on the distribution and breeding of barnacles and other intertidal animals; work is in progress on cinematographic and other techniques for studying their behaviour. The results of several years' field investigations have been published in a joint contribution with Dr D. J. Crisp in Vol. 37, No. 1, of the *Journal*. The distribution of intertidal organisms along the Channel coasts is described and discussed in some detail in relation to temperature and other environmental factors, and it is concluded that most trends in distribution can be explained by temperature factors, modified by effects of substratum and larval dispersal. An accompanying article deals with temperatures in the intertidal zone and the extremes of temperature tolerated by some intertidal animals. Work on the population dynamics of limpets and sea weeds in the Isle of Man was completed by publication of a final note in *Annual Report*, No. 68, of the Marine Biological Station at Port Erin. A review of more recent trends in intertidal zonation studies, with a discussion on the causes of zonation, is being published in *Biological Reviews*.

Dr Southward has made further observations on the deep-sea barnacle *Hexelasma*, including the examination of specimens obtained by the Cable Ship *Marie Louise Mackay* off the south-west of Ireland, and preserved material at the British Museum (Natural History). So far, living examples have been obtained only north of the Little Sole submarine promontory, by R.V. *Sarsia*, although old shell compartments were dredged up off La Chapelle Bank. Deep water dredging is a tedious process, and it is hoped to try new methods in future. More experiments on living *Hexelasma* support the previous suggestion that there is some connexion between this species and deep sea water currents.

Dr G. A. Steven has continued to make replicate trawl hauls as regularly as possible at international Station L4 in the vicinity of Plymouth in order to obtain records of short- and long-term changes in the demersal fish fauna. At the same time he is studying the biology and life-history of *Arnoglossus laterna*, *Trigla cuculus* and *Trachurus trachurus*.

Arnoglossus laterna is a small flatfish—the most abundant flatfish in the Plymouth area—that seldom exceeds 14 cm in total length. It appears to be short lived, reaching a length of 6–9 cm at 1 year, 10·5–11·5 cm at 2 years, about 12·5 cm at 3 years and just over 13 cm at 4 years. The total life span probably does not often exceed 6–7 years. Its numbers on the trawling ground remain fairly constant throughout the year.

Trigla cuculus is also present throughout the year, but may be scarce in the winter months. It enters the trawl catches as the O-group, usually by the beginning of October. It attains a total length of 11–16 cm at 1 year, 20–25 cm at 2 years, 28–32 cm at 3 years and 34–38 cm at 4 years. Its growth rate at greater ages has not yet been determined.

Trachurus trachurus is a pelagic fish which at times can also be taken near the sea floor in a trawl. Until recent years this fish was seldom caught in the trawl in the vicinity of Plymouth, but it is now fairly common except during the months of July–September. It reaches a length of 9–13 cm at 1 year, 16–19 cm at 2 years, 21–24 cm at 3 years and 25–27 cm at 4 years. Its growth rate at greater ages has not yet been determined.

In collaboration with Miss Barbara Whitaker, warden to the Lundy Island Field Society, Dr Steven has examined collections of regurgitated food pellets of shags in order to ascertain what the birds in the Lundy breeding colonies were feeding on during the breeding season. The food remains in the pellets were found to consist almost entirely of fish bones and otoliths (mostly *Ammodytes* sp.) in the breeding seasons of both 1956 and 1957. A few crustacean fragments were found, but they appeared in every instance but one to have come from the stomachs of fish eaten.

Physiology of Marine Organisms

Dr T. I. Shaw has continued his experiments upon iodine uptake by the sea-weed *Laminaria digitata*. Many agents, among them tyrosine, catechol and pyruvate, have been found to act as reversible inhibitors for ^{131}I accumulation. In every case the inhibitors have been found to prevent the weed from liberating I_2 from iodide. These findings reinforce the view that I_2, or a related oxidation state of the element, is involved in the process of iodide accumulation. The rate of uptake varies with the iodine content of the medium in a way that parallels the expected concentration of hypoiodous acid rather than I_2 and it seems likely that HIO is the form that enters the tissues.

The oxygen consumption of the weed during iodine accumulation has been shown to be too rapid, in comparison with the known diffusion constant of oxygen and the thickness of the weed, to occur uniformly throughout the tissues. It must be principally localized at the surface of the plant. Since an increase in the partial pressure of oxygen does little to hasten its consumption, the localization cannot be simply due to the slow diffusion of O_2 through the tissues.

Analyses are being made of the likely intermediates of carbohydrate metabolism in the weed, both in the presence and absence of iodide. The object is to identify the rate-limiting reaction in sugar breakdown, a reaction which must also be involved in iodine accumulation. Pyruvate, α-ketoglutarate and citrate have already been studied. The most interesting observation so far is that pyruvate decreases when iodide is supplied aerobically, but increases if I_2 is supplied anaerobically.

Many pelagic animals are luminescent and, in deeper water, they are exclusively responsible for producing the light which is used in visual perception. The majority of these animals possess well-formed eyes, and it is desirable to know how effective is this animal light in permitting vision among deep sea species. Measurements have been made of the intensity of luminescence at sea, and data are now being gathered for the light output of single animals. In furtherance of this research, Dr J. A. C. Nicol has been measuring the spectral composition and absolute intensities of the light emitted by various coastal and pelagic species, including Protozoa, jellyfish, sea-pens, ctenophores, polychaetes, *Pholas*, *Pyrosoma*, and fish. Special apparatus has been devised for analysing flashes of short duration, and thereby obtaining spectral emission curves. This apparatus has been used successfully at sea, on board R.V. *Sarsia*. Luminescence has been recorded from a number of pelagic species for the first time (various radiolarians, jellyfish and fish). Absolute values for light intensities (radiant flux) have also been secured for many species. Some representative values are as follows. *Noctiluca*, a protozoan, has a blue light, with maximal emission at 470 $m\mu$. The flux from a single cell at 1 cm is about 1×10^{-8} microjoule/1 cm^2 receptor surface. A deep-sea jellyfish, *Atolla wyvillei*, emits blue light with a peak at 470 $m\mu$; light intensities range up to $1 \times 10^{-4} \mu W\ cm^2$ receptor surface at 1 cm. Other animals emitting blue light are Radiolaria, Hydromedusae, *Chaetopterus*, *Pholas* and lantern fish (myctophids). Intensity measurements for myctophids give values up to $5 \times 10^{-4} \mu W/cm^2$ receptor surface at 1 cm. Ultimately it should be quite possible to correlate this information with that secured for the sensitivity of photoreceptor systems of marine animals. Part of this work has been published in the *Journal* (Vol. 35, pp. 529 and 629) and other parts are being prepared for publication.

Dr E. J. Denton has continued observations on vision in fishes and other marine animals. Some results of this research have been published in Vol. 36, No. 3, of the *Journal*, showing that deep-sea fish should be able to see daylight at a depth of more than 1000 m in the open ocean. At this depth light from luminescent organisms is probably already becoming predominant.

The apparatus referred to in last year's Report of Council has been modified to give absorption curves for unbleached retinae. It has been shown on a number of fishes, including deep-sea and freshwater species, that the absorption curves for whole retinae are almost the same as those given by very pure retinal extracts. It appears that most of the 'impurities' so commonly found in extracts are manufactured during extraction. It has been confirmed that the retinal densities of chrysopsin in fresh deep-sea fish are often around

1·0 for the wavelength most absorbed. A short account of this work has been published in the proceedings of the National Physical Laboratory symposium on colour vision.

In collaboration with Miss M. A. Walker of the Institute of Ophthalmology, London, Dr Denton has made a detailed comparison of the absorption of light by intact retinae and by retinal extracts of the conger eel. The retinal pigment, which is of the golden deep-sea type, is approximately twice as effective at absorbing light *in situ* than in extracts, whilst the absorption curve for the extract is shifted a few mμ towards the shorter wavelength end of the spectrum. The results of this research have been published in the *Proceedings of the Royal Society* B, Vol. 148.

Dr Denton and Dr D. B. Carlisle have found that the retina of the freshwater eel, purple in the immature form, becomes golden as the eel metamorphoses in preparation for its return to the deep sea. This golden pigment has an absorption curve identical with that of the conger eel and is typical of the retinal pigment of a deep-sea fish. The retinal density of pigment in the mature form is higher than in the immature form.

With the help of Mr F. J. Warren, Dr Denton has shown that the mid-water oceanic fish have crystalline lenses which are transparent down to 310 mμ. The majority of diurnal shallow-water fish have previously been shown to have lenses in which the light below about 400 mμ is absorbed. This may be an adaptation to improve acuity or to protect the photoproducts of bleaching against further photochemical action. Oceanic squid show similar differences in lens transparency.

Through the kindness of Dr S. K. Kon, of the Development Commission's Fisheries Biochemical Unit, Dr Denton has been able to use their fluorescence microscope at Reading. He has confirmed that the visual white (probably vitamin A) in the bleached retina of the frog remains in an organized state within the retinal rods for at least 2½ h after bleaching. The fluorescence of the molecules of visual white within the rods is polarized in such a way as to support the hypothesis that these molecules are orientated with their axes of resonance along the axes of the rods.

With Mr N. B. Marshall of the British Museum (Natural History) Dr Denton is starting a study of the biology of deep-sea fish. He has made experiments on board R.V. *Sarsia* on deep-sea fish caught alive which show that these fish can be within ½% of hydrostatic equilibrium with sea water, although they have no air-filled swim-bladder. This surprisingly high buoyancy does not appear to be achieved by any one modification but by having more fat, a more fragile and lighter skeleton, and less protein than usual. Mr E. I. Butler is assisting in the fat and protein analysis.

Experiments upon the swim-bladders of fish have been started by Dr Denton and Dr Shaw. They have developed a new method, suitable for use at sea, which gives an accurate measure of the gas content of fish *in vivo*. Using this apparatus in Plymouth and on board R.V. *Sarsia* it has been found, contrary to expectations, that fish do not necessarily secrete gas in response to an increased hydrostatic pressure. The fish, conger eels and *Diaphus rafinesquei* (Cocco), were lowered to various depths (the congers down to 1000 m) and recovered 12–72 h later. The congers lowered to 500 m or more died whilst the others failed to show any increase, indeed most showed a decrease, in gas volume.

Control of gas loss by the pneumatic duct has been studied in conger eels. It has been found that in decapitate and living fish gas escaped whenever swim-bladder volume increased to approximately twice that required for neutral buoyancy, a condition in which the swim-bladder becomes a disadvantage to the fish.

The histology and gross anatomy of the swim-bladder and related structures are also receiving attention.

Dr D. B. Carlisle has directed most of his attention this year to the endocrinology of Crustacea and continued his fruitful collaboration with Sir Francis Knowles of Marlborough. Their review of 'Endocrine control in the Crustacea' appeared in November 1956 issue of *Biological Reviews*, while a book on the subject of crustacean hormones is in the press and is to be published shortly by the Cambridge University Press as one of the series of monographs on experimental biology.

Dr Carlisle's investigations of moulting in Crustacea have this year been mainly directed at elucidating the nature of the control of the termination of moulting in crabs. The spider crab, *Maia*, is not sexually mature until it has undergone its last moult: thereafter it never moults again. The shore crab, *Carcinus*, moults 10 or 11 times after the moult of puberty and then ceases to moult. The edible crab, *Cancer*, can apparently continue to moult indefinitely. Two endocrine organs are concerned in the control of moulting: the X organ-sinus gland complex, which secretes a hormone tending to inhibit moulting, and the Y organ, which, besides a hormone necessary for the moult process to take place, also secretes a hormone responsible for the maturation of the juvenile gonad. In *Maia*, at the moult of puberty the Y-organ degenerates, as soon, that is, as the gonads are mature; thereafter moulting is no longer possible in the absence of the necessary hormone. In *Carcinus* the Y organ persists, but after the final moult the X organ produces far more moult-inhibiting hormone than at any stage earlier in the crab's life. This effectively prevents further moulting from taking place, but removal of the X organ-sinus gland complex from these crabs, after the final moult, allows moulting to begin once more with the production of 'giant' crabs. In *Cancer*, the Y organ does not degenerate nor does the X organ produce excess moult-inhibiting hormone; moulting can therefore continue without let. Some of the results of this work have been published in Vol. 36, No. 2, of the *Journal*. In connexion with this work Dr Carlisle has been employing diving equipment in an attempt to study the biology of crabs in their natural habitat. The information gained has made the interpretation of experiments easier.

On a second visit to Sweden, to attend the International Symposium on Neurosecretion, Dr Carlisle took the opportunity to extend further his investigations of the endocrine basis of sex reversal in *Pandalus*, and to take specimens of the endocrine organs of this species for histological study. While in Sweden he also began an investigation in collaboration with Prof. L. H. Kleinholz into the hormone responsible for provoking the proximal migration of the distal pigment of the eye of Natantia. This investigation was continued later in Plymouth, with Sir Francis Knowles joining in the collaboration, and led to the demonstration that the principle responsible is distinct from any of the known chromactivating substances.

Dr P. Karlson, of Munich, and Dr Carlisle have shown that a chromactivating substance active upon *Leander* and comparable to substance A of that species, is present in impure samples of ecdyson obtained from silkworms, *Bombyx* sp. It is not yet clear whether it is ecdyson itself which has this action or whether it is some impurity.

Dr Carlisle has devised a cell whereby the pituitary stalk of *Lophius* may be examined alive under the microscope when it is still attached to the pituitary and the brain. Under these conditions neurosecretory transport may be observed, particularly by the use of polarized light, and the phenomenon has been filmed. A note on this is being published in the Reports of the Second International Symposium on Neurosecretion.

Dr Abbott and Dr Carlisle have been investigating the electrophysiology of the pituitary stalk of *Lophius* and preliminary results suggest that it is capable of propagating normal action potentials.

Dr B. C. Abbott has continued experiments on the properties of invertebrate smooth muscle in collaboration with Dr J. Lowy of the Biophysics Research Unit, King's College, London. Further measurements have been made on the opacity changes which occur in the mantle muscle of squid, with emphasis on the scattered light. Polar diagrams for the forward scattering have been plotted under conditions of rest, of twitch and tetanic activity, and after death. The influence of wavelength of the light on the scattering has also been studied. Preliminary experiments have also been carried out on the properties of the funnel retractor of the squid.

The results of experiments in collaboration with Prof. A. V. Hill and Mr J. V. Howarth of University College London have been published in the *Proceedings of the Royal Society* B, Vol. 148: following a single impulse in a crab nerve at 0° C there is a rapid burst of heat, followed by the reabsorption of the majority of this heat. The initial burst of heat is comparable in value with the heat of mixing resulting from the movement of ions across the membrane during activity. Measurements have been made of the loss of potassium ions when crab nerve at low temperature is stimulated: ^{42}K exchange was measured over a range of stimulus frequencies. A value for the potassium efflux for an isolated impulse was estimated.

Experiments were also begun to find whether the crab nerve will continue for a reasonable period of time to conduct action potentials in a bathing medium where the sodium ion is replaced by lithium: this was attempted because the heat of mixing due to ion movements should then be appreciably different from that with normal sea water.

Thermal measurements on the electric organ of the electric ray *Torpedo* have been made at the Station de Biologie Marine d'Arcachon in collaboration with Prof. A. Fessard from the Collège de France, Paris, and with Dr X. M. Aubert from Louvain. Organs were isolated with their nerves and mounted in a thermostat in air between rectangular non-polarizable (Ag–AgCl) plates which were as large as the organ surface. A small thermistor was inserted into the organ and connected to a galvanometer–photocell–cathode-ray tube system. Temperature changes following stimulation were studied at various temperatures, and with different external load resistances connected between the plates. A rapid heating, larger than the internal Joule effect, was followed by a long-lasting cooling such that the overall effect was often a cooling. Control experiments showed that this effect could not be due to dead tissue. More experiments are required to be quite sure of the quantities of heat involved.

Dr E. D. S. Corner, International Paints Research Fellow, in collaboration with Mr B. W. P. Sparrow (International Paints Research Laboratory, Newton Ferrers), has completed an investigation of the factors influencing the toxicities of various mercury compounds to certain crustacea. An account of this work has been published in Vol. 36, No. 3, of the *Journal*.

This study has been continued in collaboration with Dr F. H. Rigler, of Toronto University. Tracer isotopes have been used and the experiments have shown that differences between the susceptibilities of *Artemia salina* and *Elminius modestus* to ^{203}Hg-labelled mercuric chloride and *n*-amylmercuric chloride are directly related to the rates at which the poisons are accumulated: they do not reflect the quantities of these compounds which the animals can tolerate in their tissues. Thus, mercury is taken up at approximately the same rate from equitoxic solutions of the two poisons; and the rates at which mercury is accumulated by the two species from equitoxic solutions of either poison are of the same order. Experiments in which the poisoned animals have been washed with reduced glutathione have given results consistent with the view that most of the mercury taken up by either species penetrates into the tissues of the test animals and does not act simply by becoming attached to their surfaces. Direct evidence of the penetration of mercury compounds into a crustacean has been obtained in experiments with the prawn *Leander serratus*. Both mercuric chloride and *n*-amylmercuric chloride have been detected in various organs of the poisoned animals, a large quantity of the poison being present in the antennary gland. In addition it has been found that both mercury compounds concentrate in the gills of the test animals. An account of this work has been published in Vol. 37, No. 1, of the *Journal*.

Further studies on mercury poisoning have been carried out by Dr Corner, using *Maia squinado*. In these experiments it has been found that when the animals are immersed in sea water containing ^{203}Hg-labelled mercuric chloride the poison accumulates in the blood, antennary gland, and, eventually the urine. In addition, it has been found that the urine of *Maia* poisoned with mercuric chloride contains greatly increased quantities of α-amino nitrogen. Evidence has also been obtained that the gills of the poisoned animals are heavily contaminated with mercury and it is hoped to carry out further work in order to see what effect the poison has on their normal function.

THE LIBRARY

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

The Council wish to record their thanks to Dr Mary Parke for a gift to the library of a number of important botanical works, chiefly on marine algae.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

The Library has again been much used by visiting members of the Association.

PUBLISHED MEMOIRS

Vol. 36, No. 2 of the *Journal* was published in June, Vol. 36, No. 3 in November 1957 and Vol. 37, No. 8 in February 1958.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

ABBOTT, B. C., HILL, A. V. & HOWARTH, J. V., 1958. The positive and negative heat production associated with a nerve impulse. *Proc. Roy. Soc.*, Ser. B, Vol. 148, pp. 149–87.

ALEXANDROWICZ, J. S., 1957. Notes on the nervous system in the Stomatopoda. V. The various types of sensory nerve cells. *Pubbl. Staz. zool. Napoli*, Vol. 29, pp. 213–25.

BIDDER, A. M., 1957. Evidence for an absorptive function in the 'Liver' of *Octopus vulgaris* Lam. *Pubbl. Staz. zool. Napoli*, Vol. 29, pp. 139–50.

BODEN, B. P. & KAMPA, E. M., 1957. Records of bioluminescence in the ocean. *Pacif. Sci.*, Vol. 11, pp. 229–35.

CALDWELL, P. C., 1957. The sensitivity to pH of the inhibitory effects of dinitrophenol on squid giant axons. Proc. biochem. Soc., 19 July 1957. *Biochem. J.*, Vol. 67, 1–2 P.

Caldwell, P. C. & Keynes, R. D., 1957. The utilization of phosphate bond energy for sodium extrusion from giant axons. Proc. physiol. Soc., 29–30 March 1957. *J. Physiol.*, Vol. 137, 12–13*P*.

Cooper, L. H. N., 1957. Death of a channel herring fishery. *New Scientist*, 4 pp., 2 May.

Dales, R. Phillips, 1957. Pelagic polychaetes from the Bay of Biscay. *Ann. Mag. Nat. Hist.*, Vol. 10, pp. 661–64, (12th Ser.).

Dartnall, H. J. A., 1957. The spectral variation of the photo-sensitivities of some visual pigments. *Paper* 3, *Symposium on Visual Problems of Colour held at Nat. Phys. Lab., Teddington, Middx. from 23rd to 25th September*, 1957, 26 *pp*.

Denton, E. J. & Walker, M. A., 1958. The visual pigment of the conger eel. *Proc. Roy. Soc.*, Ser. B, Vol. 148, pp. 257–69.

Frankenhaeuser, B. & Hodgkin, A. L., 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol.*, Vol. 137, pp. 218–44.

Hanson, Jean & Lowy, J., 1957. Structure of smooth muscles. *Nature, Lond.*, Vol. 180, pp. 906–9.

Hodgkin, A. L., 1958. Ionic movements and electrical activity in giant nerve fibres. The Croonian Lecture. *Proc. Roy. Soc.*, Ser. B, Vol. 148, pp. 1–37.

Hodgkin, A. L. & Keynes, R. D., 1957. Movements of labelled calcium in squid giant axons. *J. Physiol.*, Vol. 138, pp. 253–81.

Jones, W. C., 1957. The contractility and healing behaviour of pieces of *Leucosolenia complicata*. *Quart. J. micr. Sci.*, Vol. 98, pp. 203–17.

Macdonald, J. D., Pike, R. B. & Williamson, D. I., 1957. Larvae of the British species of *Diogenes*, *Pagurus*, *Anapagurus* and *Lithodes* (Crustacea, Decapoda). *Proc. zool. Soc. Lond.*, Vol. 128, pp. 209–57.

Marine Biological Association, 1957. *Plymouth Marine Fauna*, Third Edition, pp. i–xliii, 1–457, with 4 charts.

North, W. J. & Pantin, C. F. A., 1958. Sensitivity to light in the sea-anemone *Metridium senile* (L.): adaptation and action spectra. *Proc. Roy. Soc.*, Ser. B, Vol. 148, pp. 385–96.

Nicol, J. A. C., 1957. Observations on photophores and luminescence in the teleost *Porichthys*. *Quart. J. micr. Sci.*, Vol. 98, pp. 179–88.

Robson, Elaine A., 1957. The structure and hydromechanics of the musculo-epithelium in *Metridium*. *Quart. J. micr. Sci.*, Vol. 98, pp. 265–78.

Sutton, Muriel F., 1957. The feeding mechanism, functional morphology and histology of the alimentary canal of *Terebella lapidaria* L. (Polychaeta). *Proc. zool. Soc. Lond.*, Vol. 129, pp. 487–523.

Shaw, T. I. & Cooper, L. H. N., 1957. State of iodine in sea water. *Nature, Lond.*, Vol. 180, p. 250.

Stott, F. C., 1957. Observations on the food canal and associated structures in the holothurian *Holothuria forskali* Delle Chiaje. *Proc. zool. Soc. Lond.*, Vol. 129, pp. 129–36.

Membership of the Association

The total number of members on 1 March 1958 was 994, being 94 more than on 31 March 1957; of these the number of life members was 115 and of annual members 879. The number of associate members is four.

During the year Dr Å. Vedel Tåning was elected an Honorary Member.

GRANTS FOR AQUARIUM RECONSTRUCTION

The Council wish to record their grateful thanks for the following generous grants towards the cost of reconstructing the aquarium.

Nuffield Foundation	£12,000
Royal Society	£500
Imperial Chemical Industries	£200

In addition there will be a sum of approximately £500 from donations placed by the general public, over the past ten years, in the collecting box in the aquarium.

GRANT FOR CULTURE COLLECTION

The Council wish to record their thanks to the International Union of Biological Sciences for a grant of $500 towards the cost of upkeep of Dr Mary Parke's culture collection of marine micro-organisms kept at the Plymouth laboratory.

FINANCE

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Capital Grants. The Council wish to record their thanks to the Development Commissioners for a substantial capital grant to meet the cost of constructing two new sea-water reservoirs, and the building of new outside circulation tanks. They also wish to record their thanks for a grant towards the cost of publication of the third edition of the *Plymouth Marine Fauna.*

Private Income. The Council gratefully acknowledge the following generous grants received during the year:

Fishmongers' Company (£400), The Royal Society (£100), British Association (£50), Physiological Society (£50), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20), Durham (£10. 10*s*.), Manchester (£10. 10*s*.), Sheffield (£10. 10*s*.), Southampton (£15. 15*s*.), Reading (£10. 10*s*.), Nottingham (£10. 10*s*.), Hull (£10. 10*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), the Imperial College of Science and Technology (£10), Gonville and Caius College, Cambridge (£5), and the Zoological Society of London (£10. 10*s*.).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1958–59:

President

Prof. A. V. Hill, C.H., O.B.E., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, K.G., C.B., C.M.G.
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Sir Edward J. Salisbury, Kt., C.B.E., D.Sc., F.R.S.
A. T. A. Dobson, C.B., C.V.O., C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Earl of Verulam
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.

COUNCIL

To retire in 1959

G. E. R. Deacon, C.B.E., D.Sc., F.R.S.
M. N. Hill, Ph.D.
O. D. Hunt
Prof. R. J. Pumphrey, Sc.D., F.R.S.
Prof. G. P. Wells, Sc.D., F.R.S.

To retire in 1960

Prof. E. Baldwin, Ph.D.
C. H. Mortimer, Dr.phil., D.Sc., F.R.S.
C. F. A. Pantin, Sc.D., F.R.S.
Prof. J. E. Smith, Sc.D., F.R.S.
H. G. Vevers, M.B.E., D.Phil.

To retire in 1961

H. A. Cole, D.Sc.
G. E. Fogg, Ph.D.
Prof. J. E. Harris, Ph.D., F.R.S
C. E. Lucas, C.M.G., D.Sc.
Prof. C. M. Yonge, C.B.E., D.Sc., F.R.S.

Hon. Treasurer

Harrison S. Edwards, Westhumble Lacey, Near Dorking, Surrey

Secretary

F. S. Russell, C.B.E., D.S.C., D.F.C., LL.D., F.R.S.

The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of Council:

R. G. R. Wall (Ministry of Agriculture, Fisheries and Food)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. Christie-Miller
  Harrison S. Edwards
Prof. Sir Alister Hardy, Kt., D.Sc., F.R.S. (Oxford University)
S. Smith, Ph.D. (Cambridge University)
Edward Hindle, Sc.D., F.R.S. (British Association)
N. B. Marshall (Zoological Society)
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 MARCH 1958

	£	£
CAPITAL RESERVE ACCOUNT:		
As at 31 March 1957	176,084	
Add: Expenditure on fixed assets recovered	1,670	
	177,754	
Less: Transfer to surplus account being an amount equivalent to the depreciation provided on assets acquired out of Development Fund grants	3,700	174,054
SURPLUS ACCOUNT:		
As at 31 March 1957	6,782	
Add: Excess of income over expenditure for the year	3,160	
Transfer from Capital Reserve Account	3,700	
	13,642	
Deduct: Increase in provision for diminution in value of General Fund investments	120	13,522
		187,576
BALANCES ON SPECIAL FUNDS (see annexed statement)		7,669
CURRENT LIABILITIES:		
Sundry creditors and accrued expenses	1,997	
Subscriptions and grants received in advance	184	2,181
		£197,426

Note: Capital commitments outstanding amount to £1,200 (1957 £10,400) no part (1957 £9,300) of which will be recoverable under Development Fund grants.

M. N. HILL } *Members of the Council*
O. D. HUNT }

	Cost or Valuation £	Depreciation £	£
FIXED ASSETS:			
Boats and equipment:			
At cost:			
R.V. 'Sarsia'	137,761	3,270	134,491
M.F.V. 'Sula'	12,500	420	12,080
R.L. 'Gammarus'	200	10	190
	150,461	3,700	146,761
Laboratory apparatus, equipment and machinery:			
At cost	16,169	4,019	12,150
Library at valuation in 1941 plus additions as valued by the Director	23,100	—	23,100
	£189,730	£7,719	
			182,011
INVESTMENTS:			
General Fund (including Composition Fees) at book amount (Market value £1,220; last year £1,277)		1,672	
E. T. Browne Bequest Funds at cost (Market value £3,086; last year £3,346)		4,582	
		6,254	
Less: Provision for diminution in value of investments		1,948	4,306
CURRENT ASSETS:			
Stocks on hand as valued by the Director		3,467	
Sundry debtors and prepayments		4,470	
Balances at bankers and cash in hand		3,172	11,109
			£197,426

AUDITORS' REPORT TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31 March 1958 and of its excess of income over expenditure for the year ended on that date.

We have obtained all the information and explanations which we considered necessary. In our opinion the Association has kept proper books and the said accounts which are in agreement with them and with the said information and explanations, give in the prescribed manner the information required by the Companies Act 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
16 *May* 1958

PRICE WATERHOUSE & CO.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 MARCH 1958

	£	£
SALARIES (including additional for previous year) NATIONAL INSURANCE AND SUPERANNUATION SCHEME CONTRIBUTIONS		35,788
LABORATORY AND BOATS' CREWS' WAGES (including additional for previous year), NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS AND EMPLOYERS' LIABILITY INSURANCE		32,593
UPKEEP OF LIBRARY		874
SCIENTIFIC PUBLICATIONS, *less* SALES		2,255
UPKEEP OF LABORATORIES:		
Buildings and machinery	827	
Electricity, gas, coal and water	1,133	
Chemicals and apparatus	1,761	
Depreciation of Laboratory apparatus, equipment and machinery	1,351	
Rents and insurance	295	
Travelling expenses	833	
Audit fee	138	
Stationery, postage, telephone and sundries	1,192	
Specimens	225	
Collecting expenses and upkeep of truck	235	
		7,990
MAINTENANCE AND OPERATION OF BOATS:		
Petrol, oil, paraffin, etc.	1,549	
Maintenance and repairs	6,905	
Depreciation	3,700	
Insurances	2,460	
Hire of Decca Navigator—R.V. 'Sarsia'	395	
		15,009
ENTERTAINMENT EXPENSES		50
BALANCE being excess of income over expenditure for the year		3,160
		£97,719

	£	£	£
GRANTS AND TABLE RENTS:			
Ministry of Agriculture, Fisheries and Food Grant from Development Fund		88,703	
Fishmongers' Company		400	
Miscellaneous (including Royal Society £100, British Association £50, Physiological Society £50, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Southampton £15. 15*s*., Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Reading £10. 10*s*., and Sheffield £10. 10*s*., Imperial College, £10, Zoological Society of London £10. 10*s*., Ministry of Works £96, Imperial Chemical Industries Ltd. £52. 10*s*., International Paints Ltd. £52. 10*s*., Gonville and Caius College, Cambridge £5)		1,267	
			90,370
SUBSCRIPTIONS			856
SALES:			
Specimens		2,971	
Fish		712	
	£		
Nets, gear and hydrographical apparatus	715		
Less: Cost of materials	366		
		349	
			4,032
SUNDRY PUBLICATIONS			291
INCOME FROM INVESTMENTS			55
INTEREST ON BANK DEPOSITS, *less* CHARGES			182
AQUARIUM:			
Admission Fees		2,124	
Sale of guides		47	
		2,171	
Less: Maintenance, printing and advertising		238	
			1,933
			£97,719

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31 MARCH 1958

	Aquarium Reconstruction Fund	E. T. Browne Bequest			Rockefeller Foundation Fund	Library Extension and New Dogfish House Fund	'Plymouth Marine Fauna' Fund	Reservoir and Sea Water Tanks Fund	Algal Culture Fund	Research Funds*	TOTAL
		Library	Special Apparatus	Scientific Publications							
	£	£	£	£	£	£	£	£	£	£	£
BALANCE AT 31 MARCH 1957 (after providing £1,151 for diminution in value of investments)	431	1,051	2,052	464	269	1,187	(3)	1,163	—	288	6,902
Add: Income during year											
Grants	700	—	—	—	2,631	—	500	8,330	178	3,571	15,910
Income from investments	—	40	83	21	—	—	—	—	—	—	144
Bank deposit interest	15	—	—	—	—	—	—	33	—	—	48
Other income	26	—	—	85	—	—	—	—	—	—	111
Stock of 'Plymouth Marine Fauna' at 31 March 1958	—	—	—	—	—	—	1,100	—	—	—	1,100
Transfer between Special Funds	—	—	—	—	(125)	—	125	—	—	—	—
	1172	1,091	2,135	570	2,775	1,187	1,722	9,526	178	3,859	24,215
Deduct: Expenditure during year	—	31	82	—	2,761	508	622	8,692	—	3,505	16,201
Increase in provision for diminution in value of investments	—	85	218	42	—	—	—	—	—	—	345
BALANCE AT 31 MARCH 1958	£1,172	£975	£1,835	£528	£14	£679	£1,100	£834	£178	£354	£7,669

* Including International Paints Ltd. Research Fellowship.

LIST OF GOVERNORS, FOUNDERS, MEMBERS, HONORARY AND ASSOCIATE MEMBERS

1958

GOVERNORS

THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, Burlington House, W. 1
THE UNIVERSITY OF OXFORD
THE UNIVERSITY OF CAMBRIDGE
THE WORSHIPFUL COMPANY OF CLOTHWORKERS, 48 Fenchurch Street, E.C. 3
THE WORSHIPFUL COMPANY OF FISHMONGERS, London Bridge, E.C. 4
  THE PRIME WARDEN. (**Council,** 1886→)
  EDWARDS, HARRISON S., Westhumble Lacey, near Dorking, Surrey. (**Council,** 1950→; **Hon. Treasurer,** 1956→)
  CHRISTIE-MILLER, Major E. G., 38 Hyde Park Street, W. 2. (**Council,** 1941→; **Hon. Treasurer,** 1941→56; **Vice-President,** 1951→)
THE ZOOLOGICAL SOCIETY OF LONDON, Regent's Park, N.W. 1
THE ROYAL SOCIETY, Burlington House, Piccadilly, W. 1
MINISTRY OF AGRICULTURE, FISHERIES & FOOD, 3 Whitehall Place, S.W. 1
BAYLY, ROBERT (the late). (**Council,** 1896–1901)
BAYLY, JOHN (the late)
BROWNE, E. T. (the late). (**Council,** 1913–19; 1920–37)
THOMASSON, J. P. (the late). (**Council,** 1896–1903)
BIDDER, G. P., Sc.D. (the late). (**Council,** 1899–1953; **President,** 1939–45; **Vice-President,** 1948–53)
THE LORD MOYNE, P.C., D.S.O. (the late). (**Vice-President,** 1929; 1939–45; **President,** 1930–39)
ALLEN, E. J., C.B.E., D.Sc., LL.D., F.R.S. (the late) (Honorary). (**Council,** 1895–1942; **Secretary,** 1895–1936; **Hon. Governor,** 1937–42)

FOUNDERS

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1884 THE WORSHIPFUL COMPANY OF MERCERS, Mercers' Hall, 4 Ironmonger Lane, E.C. 2
1884 THE WORSHIPFUL COMPANY OF GOLDSMITHS, Goldsmiths' Hall, Foster Lane, E.C. 2
1884 THE ROYAL MICROSCOPICAL SOCIETY, B.M.A. House, Tavistock Square, W.C. 1
1884 BULTEEL, THOS. (the late)
1884 BURDETT-COUTTS, W. L. A. BARTLETT (the late)
1884 CRISP, Sir FRANK, Bart. (the late). (**Council,** 1884–92; **Hon. Treasurer,** 1884–88)
1884 DAUBENY, Captain GILES A. (the late)
1884 EDDY, J. RAY (the late)
1884 GASSIOT, JOHN P. (the late)
1884 LANKESTER, Sir E. RAY, K.C.B., F.R.S. (the late). (**Hon. Secretary,** 1884–90; **President,** 1891–1929)

1884 Lord MASHAM (the late)
1884 MOSELEY, Prof. H. N., F.R.S. (the late). (**Chairman of Council**, 1884–88)
1884 Lord AVEBURY, F.R.S. (the late). (**Vice-President**, 1884–1913)
1884 POULTON, Prof. Sir EDWARD B., F.R.S. (the late). (**Council**, 1888–94)
1884 ROMANES, Prof. G. J., LL.D., F.R.S. (the late). (**Council**, 1884–91)
1884 WORTHINGTON, JAMES (the late)
1885 The 15th EARL OF DERBY (the late)
1887 WELDON, Prof. W. F. R., F.R.S. (the late). (**Council**, 1890–1901; representing British Association, 1901–5)
1888 BURY, HENRY, (the late)
1888 THE WORSHIPFUL COMPANY OF DRAPERS, Drapers' Hall, E.C. 2
1889 THE WORSHIPFUL COMPANY OF GROCERS, Grocers' Hall, Princes Street, E.C. 2
1889 THOMPSON, Sir HENRY, Bart. (the late). (**Vice-President**, 1890–1903)
1889 Lord REVELSTOKE (the late)
1890 RICHES, T. H. (the late). (**Council**, 1920–25)
1892 BROWNE, Mrs E. T. (the late)
1898 WORTH, R. HANSFORD, M.Inst.C.E. (the late)
1899 The EARL OF IVEAGH, K.G., C.B., C.M.G., 11 St James's Square, S.W. 1 (**Vice-President**, 1929→)
1902 GURNEY, ROBERT, D.Sc. (the late). (**Council**, 1932–5)
1904 SHAW, JOSEPH, K.C. (the late)
1909 HARDING, Colonel W. (the late)
1910 MURRAY, Sir JOHN, K.C.B., F.R.S. (the late). (**Council**, 1896–99; **Vice-President**, 1900–13)
1912 SWITHINBANK, H. (the late)
1913 SHEARER, Dr CRESSWELL, F.R.S. (the late)
1913 HERON-ALLEN, E., F.R.S. (the late)
1918 EVANS, GEORGE (the late). (**Hon. Treasurer**, 1915–31; **Vice-President**, 1925–33)
1920 MCCLEAN, Capt. W. N., 39 Phillimore Gardens, W. 8
1920 Lord BUCKLAND OF BWLCH (the late)
1920 LLEWELLYN, Sir D. R. (the late)
1921 HARMER, F. W. (the late)
1924 THE MACFISHERIES, LTD., Ocean House, Pudding Lane, E.C. 3
1924 Lady MURRAY (the late)
1925 THE INSTITUTION OF CIVIL ENGINEERS, Great George Street, Westminster, S.W. 1
1925 DISCOVERY COMMITTEE
1927 BIDDER, Miss ANNA M., Ph.D., 2A Cavendish Avenue, Hills Road, Cambridge. (**Council**, 1948–51, 1954–57)
1933 PEEL, Col. Sir EDWARD T., K.B.E., D.S.O., M.C., c/o Messrs Peel and Co., Ltd. P.O. Box 331, Alexandria, Egypt. (**Vice-President**, 1936→)
1938 BUCHANAN, Dr FLORENCE (the late)
1945 BROWN, ARTHUR W. W. (the late)

MEMBERS

* Life Members

1949 ABBOTT, B. C., Ph.D., F.Inst.P., Dept. of Zoology, University of California, Los Angeles 24, Calif., U.S.A.
1945 ABERDEEN UNIVERSITY LIBRARY, The University, Aberdeen
1934 ADAM, Mrs K. M. G., 84 Lasswade Road, Edinburgh 9

1951 Adams, E., 2 Woodford Crescent, Marsh Mills, Plympton, Devon
*1954 Adams, Miss M. N. E., 11 Milner Road, Kingston-on-Thames, Surrey
1957 Adcock, N. W., Rossignol, Harlaxton Drive, Long Eaton, Notts
1940 Lord Adrian, O.M., M.D., D.Sc., LL.D., F.R.S., The Master's Lodge, Trinity College, Cambridge
1947 Affleck, R. J., 1 Helmsdale Road, London, S.W. 16
1957 Agrawal, V. P., Dept. of Zoology, Queen Mary College, Mile End Road, London, E. 1
1957 Akkeshi Marine Biological Station: Akkeshi, Hokkaido, Japan
1950 Alexandrowicz, J. S., Ph.D., M.D., The Laboratory, Citadel Hill, Plymouth, Devon
1957 Allen, Miss D. M., Cedars, Furzedown College, Welham Road, Tooting, London, S.W. 17
1954 Allen, G. L., The Nook, 87A Bury Old Road, Sedgley Park, Prestwich, Manchester
1951 Allen, J. A., Dove Marine Laboratory, Cullercoats, Northumbs
1952 Allen, Miss J. M., Tenements Farm, Chipperfield, Herts
1953 Alvariño, Señora A., Doce de Octubre 11–1 °C, Madrid, Spain
*1927 Amirthalingam, C., Ph.D., 2 Dickmans Path, Colombo, Ceylon
1957 Angus, L. H., A.R.I.C., Three Gables, Torridge, Plympton, Plymouth, Devon
1956 Ansell, A. D., 22 Mannion Road, Henley-on-Thames, Oxon
1950 Arnold, D. C., Gatty Marine Laboratory, The University, St Andrews, Fife
1944 Ashby, D. G., c/o P.O. Box 61, Stellenbosch, Cape Province, S. Africa
1954 Ashhurst, Miss D. E., Heron Court, Alexandra Road, Epsom, Surrey
1958 Atherton, D., Ph.D., J. S. Craig & Co. Ltd., 87 Portman Street, Glasgow, S. 1
1957 Aston, C. E. J., 23 Beechcroft Avenue, Harrow, Middx
*1929 Atkins, Miss D., D.Sc., c/o The Laboratory, Citadel Hill, Plymouth, Devon
*1939 Atkins, W. R. G., C.B.E., O.B.E. (mil.), Sc.D., F.R.I.C., F.Inst.P., F.R.S., The Old Vicarage, Antony, Torpoint, Cornwall
*1910 Atkinson, G. T., Gresham House, Esplanade, Lowestoft, Suffolk
1951 Atlantic Biological Station, St Andrews, N.B., Canada

1948 Baal, H. J., 3 Bel Royal Villas, Jersey, C.I.
1957 Baer, Prof. J. G., Institut de Zoologie, Rue Emile Argand, Neuchâtel, Switzerland
1950 Baerends, Prof. G. P., Zoölogisch Laboratorium, Rijksstraatweg 78, Haren (Gron.), Holland
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1956 Bailey, Miss J. A., 110 Cambridge Road, North Harrow, Middx
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1950 Bainbridge, R., Ph.D., 43 Strathmore Avenue, Hull
1953 Bainbridge, V., Fisheries Laboratory, Lowestoft, Suffolk
1957 Baker, C. J. E., 34 St John's Road, Oxford
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1936 Baldwin, Prof. E., Ph.D., Dept of Biochemistry, University College, Gower Street, London, W.C. 1 (**Council**, 1946–48, 1957→)
1955 Ballantine, W. J., A3 Downing College, Cambridge
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1956 Barnard, J. Laurens, Ph.D., Allan Hancock Foundation, University of California, Los Angeles 7, Calif., U.S.A.
1939 Barnes, H., D.Sc., F.R.I.C., Marine Station, Millport, Isle of Cumbrae, Scotland
1954 Barnes, M. McC., Mandeville, Rosebank Crescent, Pennsylvania, Exeter, Devon
1955 Barnett, P. R. O., Cloudshill, Eaton Avenue, Allestree, Derby
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1939 Barrington, Prof. E. J. W., D.Sc., Dept. of Zoology, The University, Nottingham
1951 Barron, H., 65 Sumerton Road, Belfast, N. Ireland
1939 Bassindale, R., Dept. of Zoology, The University, Bristol
1946 Batham, Miss E. J., Ph.D., Portobello Marine Biological Station, Portobello, Otago, New Zealand
1939 Baxter, E. W., Biology Dept., Medical School, Guy's Hospital, London, S.E. 1
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1934 Beadle, L. C., Dept. of Biology, University College of East Africa, P.O. Box 262, Kampala, Uganda
1955 Beall, I. D., 19 Beresford Street, Stoke, Plymouth, Devon
1957 Beard, D. M. MacG., 123 Northcote Road, Downend, Bristol
1928 Beer, Sir Gavin De, Kt., D.Sc., F.R.S., British Museum (Natural History), Cromwell Road, London, S.W. 7
1955 Beeson, Miss G., Redgate, 216 Unthank Road, Norwich, Norfolk
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1950 Bell, Mrs E. B., Solva, Glanford Road, Brigg, Lincs
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1957 Bennett, D. P., 15 Pickering Road, Cheltenham, Glos
1954 Berner, L. D. Jr., Scripps Institution of Oceanography, La Jolla, Calif., U.S.A.
1958 Bernhard, Dr Michael, c/o Istituto di Genetica, Universita di Pavia, Pavia, Italy
1947 Berrill, Prof. N. J., F.R.S., Dept. of Zoology, McGill University, Montreal, Canada
1955 Berry, R. J., Group for Experimental Research in Inherited Diseases, University College, Gower Street, London, W.C. 1
1947 Best, A. C. G., The Laboratory, Citadel Hill, Plymouth, Devon
1953 Bhattacharyya, Dr R. N., 44/b Kalighat Road, Calcutta 26, India
1956 Bhavanarayana, P. V., Zoological Laboratories, Andhra University, Waltair, S. India
1953 Bhimachar, B. S., D.Sc., F.N.I., Central Inland Fisheries Research Station, 47/1 Strand Road, Calcutta 7, India
1958 Bhupendra, P., Marine Biology Station, Menai Bridge, Anglesey
1903 Bidder, Col. H. F., The Malting House, Nettlebed, near Henley-on-Thames, Oxon
*1945 Bingley, F. J., Flatford Mill Field Centre, East Bergholt, near Colchester, Essex
1955 Binyon, E. J., 44 Kendall Avenue South, Sanderstead, Surrey
1957 Bieri, R., Ph.D., 116 Glen Street, Yellow Springs, Ohio, U.S.A.
1925 Birkbeck College, The Library, Malet Street, London, W.C. 1
1951 Birkett, L., Fisheries Laboratory, Lowestoft, Suffolk

1947 Bishop, M. W. H., Meadow Farm, Waterbeach, Cambs
1951 Blackburn, Prof. M., D.Sc., Scripps Institution of Oceanography, La Jolla, Calif., U.S.A.
1957 Blake, J. W., Dept. of Zoology, University of North Carolina, Chapel Hill, North Carolina, U.S.A.
1955 Blake, M. L., Picket Piece, Avening, near Stroud, Glos
1930 Blaschko, Dr H., Dept. of Pharmacology, South Parks Road, Oxford
1952 Blaxter, J. H. S., Marine Laboratory, Victoria Road, Torry, Aberdeen
1957 Blood, M. J., Ravenscourt, Coopers Green, Uckfield, Sussex
1955 Bloom, Miss J. L., 12 Belsize Square, Hampstead, London, N.W. 3
1910 Bloomer, H. H., Longdown, Sunnydale Road, Swanage, Dorset
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1957 Bocks, Miss S. M., 29 Milford Street, London, S.W. 8
1951 Boden, B. P., Ph.D., Scripps Institution of Oceanography, La Jolla, Calif., U.S.A.
1947 Boëtius, Dr Jan, Fysiologisk Laboratorium, Danmarks Akvarium, Charlottenlund, Denmark
1936 Bogue, Prof. J. Yule, D.Sc., Heyscroft, Hartley Road, Altrincham, Cheshire
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1957 Bond, Miss A. J., 51 Coleridge Vale Road North, Clevedon, Som
1945 Boney, A. D., 24 Ford Park Road, Mutley, Plymouth, Devon
1954 Bonham, Dr K., Applied Fisheries Laboratory, University of Washington, Seattle, Wash., U.S.A.
1957 Boolootian, R. A., Ph.D., Dept. of Zoology, University of California, Los Angeles 24, Calif., U.S.A.
1957 Booth, Miss M. A., 21 Pinfold Lane, Penn, Wolverhampton
*1933 Boschma, Prof. Dr H., Rijksmuseum van Natuurlijke Historie, Leiden, Holland
1947 Bossanyi, J., 22 Field End Road, Eastcote, Pinner, Middx
1954 Bowers, A. B., Marine Biological Station, Port Erin, Isle of Man
1955 Bradley, D. J., 71 Linden Drive, Evington, Leicester
*1954 Bradshaw, J. S., P.O. Box 891, Del Mar, Calif., U.S.A.
1957 Brailsford, J. D., 328 London Road, Wokingham, Berks
1940 Brambell, Prof. F. W. Rogers, D.Sc., F.R.S., Dept. of Zoology, University College of North Wales, Bangor, Caern. (**Council**, 1944–47, 1948–51)
1954 Brehaut, R. N., La Canurie, Collings Road, St Peter Port, Guernsey, C.I.
1924 Brightwell, L. R., East Cliff Cottage, West Portholland, Portloe, Truro, Cornwall
1958 Brimson, P. W., The Rectory, Old Cleeve, Minehead, Som
1933 Bristol University, Dept. of Zoology, Bristol
*1941 British Celanese Ltd., Celanese House, Hanover Square, London, W. 1
1939 British Ropes Ltd., Western Avenue, Cardiff
1958 British Sub-Aqua Club, Taunton Branch, The Secretary, Two Lilac Cottage, Trull, Taunton, Som
1955 British Sub-Aqua Club, Scientific Section, The Close, Newport Pagnell, Bucks
*1946 Brock, Mrs C. H., Ph.D., 32 Barton Road, Cambridge
1946 Brough, Prof. James, D.Sc., Dept. of Zoology and Comparative Anatomy, University College, Newport Road, Cardiff
1955 Brown, C. A., 26 Sydney Road, Newquay, Cornwall
1928 Brown, Miss E. M., Ph.D., 6 Effingham Lodge, Surbiton Crescent, Kingston-on-Thames, Surrey

1936 Brown, Herbert H., O.B.E., Ph.D., Fisheries Division, F.A.O.U.N., Viale delle Terme di Caracalla, Rome, Italy
1955 Bruce, Dr A. J., 24 Croftfoot Road, Glasgow, S. 4
*1954 Brull, Prof. L., Institut de Clinique et de Policlinique Médicales, Hôpital de Bavière, Université de Liége, Liége, Belgium
1953 Buchsbaum, Prof. R., Ph.D., Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pennsylvania, U.S.A.
1953 Buckee, R. E., 32 Weston Drive, Stanmore, Middx
1958 Bull, Miss F. M., Girton College, Cambridge
*1925 Bull, Herbert O., D.Sc., Dove Marine Laboratory, Cullercoats, Northumbs
1955 Burch, J. Q., 1584 West Vernon Avenue, Los Angeles 62, Calif., U.S.A.
1951 Burgess, G. H. O., D.S.I.R., Humber Laboratory, Wassand Street, Hull
1954 Burley, Miss E. A. M., Avondale, 2 Narborough Road South, Leicester
1948 Burrows, Mrs E. M., Ph.D., Hartley Botanical Laboratories, The University, Liverpool 3
1954 Burt, Wayne V., Ph.D., Associate Prof. of Oceanography, Oregon State College, Corvallis, Oregon, U.S.A.
1947 Burton, R. F., Pinecroft, Ockham Road North, East Horsley, Surrey
1949 Bush, Prof. S. F., D.Phil., Dept. of Zoology, University of Natal, Pietermaritzburg, S. Africa
1949 Butcher, A. W., Three Salmons Hotel, Usk, Mon

1955 Caldwell, P. C., D.Phil., The Laboratory, Citadel Hill, Plymouth, Devon
1955 Caldwell, Mrs P. C., c/o The Laboratory, Citadel Hill, Plymouth, Devon
1957 Callow, Mrs I. C., 12 Newport Street, Millbrook, near Plymouth
1949 Cameron, H. D., Bramley Tor, 43 Seymour Park, Mannamead, Plymouth
*1950 Cameron, Dr W. M., Director of Scientific Services, Royal Canadian Navy, Ottawa, Ontario, Canada
1920 Cannon, Prof. H. Graham, Sc.D., F.R.S., Dept. of Zoology, Victoria University, Manchester. (**Council**, 1927–30, 1932–34, 1937–41, 1942–45, 1952–55)
1954 Canterbury Museum, Rolleston Avenue, Christchurch, New Zealand
1950 Capstick, C. K., Ph.D., The United Steel Companies Limited, Dept. of Operational Research & Cybernetics, Cybor House, 1 Tapton House Road, Sheffield, 10
1958 Carr, M. R., 86 Daybrook Road, Merton Park, London, S.W. 19
1953 Carr-Saunders, E. M., Bridge House, St John's Vale, Keswick, Cumbs
1956 Carrie, B. G. A., Carisbrooke, Milverton Road, Totton, Hants
1927 Carruthers, J. N., D.Sc., F.Inst.P., National Institute of Oceanography, Wormley, near Godalming, Surrey. (**Council**, 1948–51, 1953–56)
1923 Carter, G. S., Ph.D., Dept. of Zoology, Downing Street, Cambridge
1957 Caspers, Prof. Dr H., Zoologisches Staatsinstitut, Bornplatz 5, Hamburg 13, Germany
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*1948 Cattley, J. G., Fisheries Laboratory, Lowestoft, Suffolk
1949 Centre de Recherches et d'Etudes Océanographiques, 1 Quai Branly, Paris VII, France

1955 Ceurvels, A. Russell, 1054 Hyde Park Avenue, Hyde Park 36, Mass., U.S.A.
*1949 Chang, H. W., Zoological Institute, Academia Sinica, 320 Yo Yang Road, Shanghai, China
1957 Changeux, Jean-Pierre, 45 rue d'VIm, Paris V^{e}, France
*1952 Chaplin, Viscount, Wadstray House, Blackawton, near Totnes, Devon
*1949 Chapman, Garth, Ph.D., Nunns, Coxtie Green, Brentwood, Essex
1953 Chapman, Mrs R. F., Dept. of Zoology, University College of Ghana, Achimota, Ghana
1936 Charterhouse School, Biological Dept., Godalming, Surrey
1958 Chatfield, C. I., 96 Weston Park Road, Peverell, Plymouth, Devon
1947 Cheng, Prof. Chung, Ph.D., Dept. of Oceanography, National Amoy University, Amoy, China
*1947 Chidambaram, K., Fisheries Biological Station, West Hill Post, South Malabar, India
1952 Childs, P., F.R.C.S., 8 The Crescent, Plymouth, Devon
1953 Chile: Dr Parmenio Yañez Andrade, Director, Marine Biological Station of the University of Chile, Casilla 13-D, Viña del Mar, Chile
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1946 Chipperfield, Philip N. J., Ph.D., Hillside, Manor Road, Brixham, Devon
1942 Christie-Miller, Major E. G., 38 Hyde Park Street, London, W. 2. (**Council**, 1941→; **Hon. Treasurer**, 1941→56; **Vice-President**, 1951→)
1957 Clapham, Miss J. M., 8 Claremont Place, Sheffield, 10
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1952 Clark, R. B., Dept. of Zoology, The University, Bristol
1949 Clarke, K. U., Dept. of Zoology, The University, Nottingham
1951 Clarke, Mrs M., Cumbrae, Morpeth, N.S.W., Australia
1944 Clarke, Robert H., National Institute of Oceanography, Wormley, near Godalming, Surrey
1951 Clay, Miss E., Green Hollow, Gattery Lane, Brixham, Devon
1936 Clothier, Peter, Hill Close, Street, Som
1939 Clowes, A. J., Division of Fisheries, Beach Road, Sea Point, Cape Town, S. Africa
*1886 Coates and Co. (Plymouth) Ltd., Black Friars Distillery, Southside Street, Plymouth, Devon
*1945 Cobham, Lt.-Cdr. A. J., R.N., Noel Cottage, Castle Street, Porchester, Hants
*1925 Cockshott, Lt.-Col. A. M., R.A.S.C., Cotteswold Naturalists' Field Club, City Library, Gloucester
1933 Cole, H. A., D.Sc., Fisheries Laboratory, Lowestoft, Suffolk. (**Council**, 1958→)
1955 Coles, J. W., 70 Bridgewood Road, Worcester Park, Surrey
*1948 Collier, Albert, 1009 Church Street, Galveston, Texas, U.S.A.
*1885 Collier and Co., 53 Southside Street, Plymouth, Devon
1952 Collinge, C. F., The Mount, 37 Derry Downs, Orpington, Kent
1954 Collings, D. J., 46 Belgrave Road, Mutley, Plymouth, Devon
1955 Collins, R. G., Goldsithney, near Penzance, Cornwall
1947 Collis, Miss M. M., 27 Mowbray Road, Cambridge
1930 Colman, J. S., Marine Biological Station, Port Erin, Isle of Man. (**Council**, 1951–54)
1956 Conroy, D. A., Newby, 5 Westlands Road, Copthorne, Shrewsbury
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*1933 Cooper, L. H. N., D.Sc., F.R.I.C., The Laboratory, Citadel Hill, Plymouth, Devon
1955 Cooper, R., 33 Fanshawe Street, Bengeo, Hertford, Herts
1956 Copenhagen: Universitetets Zoofysiologiske Laboratorium, Juliane Maries Vej 32, Copenhagen, Denmark
1937 Corbin, P. G., The Laboratory, Citadel Hill, Plymouth, Devon
1937 Corbin, Mrs P. G., Ph.D., Dostabrook, Horrabridge, Devon
1954 Corcoran, E. F., Marine Laboratory, 1 Rickenbacker Causeway, Virginia Key, Miami 49, Florida, U.S.A.
1946 Corlett, John, Fisheries Laboratory, Lowestoft, Suffolk
1937 Cosway, C. A., 177 Windsor Road, Torquay, Devon
1941 Cott, H. B., D.Sc., University Museum of Zoology, Cambridge
1957 Courtney, Miss W. A. M., 6 Therapia Lane, W. Croydon, Surrey
1958 Cowey, C. B., National Institute for Research in Dairying, Shinfield, Reading, Berks
1952 Cowper, T. R., C.S.I.R.O., Fisheries Division, Cronulla, N.S.W., Australia
1956 Cox, W. D. G., 5 Elm Terrace, Ridgeway, Plympton, S. Devon
1958 Coxhead, P., 12 Cambridge Square, London, W. 2
1936 Crawford, G. I., 18 East Drive, Carshalton Beeches, Surrey
1952 Crawshaw, K. Ridgway, Heron Cottage, White Notley, Witham, Essex
1953 Creek, Miss Gwen (Mrs J. M. Grant), Whitethorns, Denham Lane, Chalfont St Peter, Bucks
*1928 Crew, Prof. F. A. E., M.D., D.Sc., F.R.S., Frant Hill Lodge, 19 Frant Road, Tunbridge Wells, Kent
*1952 Crisp, D. J., Ph.D., Bryn Hydd, Holyhead Road, Bangor, Caern. (**Council**, 1954–57)
1957 Crockford, G. W., The Bystander, Sandleigh, Abingdon, Berks
1957 Croft, C. J., Lexden, Hartley Avenue, Plymouth, Devon
1929 Crofts, Miss D. R., D.Sc., Deerbank, Norsey Wood, Billericay, Essex
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1954 Curtis, A. S. G., 1 Hornton Street, London, W. 8
1950 Cushing, D. H., D.Phil., Fisheries Laboratory, Lowestoft, Suffolk
1958 Custance, D. R. M., Elton Hall, Ludlow, Salop
1955 Cutcliffe, A. S., Tranmere Hotel, St James Place, Ilfracombe, Devon
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1958 Daisley, K. W., D.Phil., Nutrition Dept., National Institute for Research in Dairying, Shinfield, Reading, Berks
1952 Dale, E., 6 Garforth Cliff Estate, Selby Road, Garforth, Leeds
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1947 Dall, William, C.S.I.R.O., Division of Fisheries, Marine Biological Laboratory, Cronulla, N.S.W., Australia
*1919 Damant, Capt. G. C. C., C.B.E., R.N., Thursford, Cambridge Road, East Cowes, I. of W. (**Council**, 1928–31, 1937–40)
1958 Dandy, J. W. T., Zoology Dept., University College of Fort Hare, P.O. Fort Hare, Cape Province, S. Africa

1939 Danielli, Prof. J. F., D.Sc., F.R.S., Dept. of Zoology, King's College, Strand, London, W.C. 2. (**Council**, 1944–45)
1954 Dare, P. J., 30 Aylesbury Crescent, Whitleigh, Plymouth, Devon
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1958 Davies, P. S., 18 Windsor Terrace, Penarth, Glam
1931 Dawes, B., D.Sc., Dept. of Zoology, King's College, Strand, London, W.C. 2
1955 Dawson, E. W., Zoological Laboratory, Downing Street, Cambridge
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1944 Day, Prof. J. H., D.F.C., Dept. of Zoology, University, Rondebosch, Cape Town, S. Africa
1938 Deacon, G. E. R., C.B.E., D.Sc., F.R.S., Flitwick House, Milford, Surrey. (**Council**, 1946–49, 1950–53, 1956→)
1957 Deakin, Miss P. A., St Hugh's College, Oxford
1952 Deevey, Miss Georgiana B., Ph.D., The Bingham Oceanographic Laboratory, Yale University, New Haven, Conn., U.S.A.
1952 Delaware: Dept. of Biological Sciences, University of Delaware, Newark, Delaware, U.S.A.
1939 Dennell, Ralph, D.Sc., Dept. of Zoology, The University, Manchester, 13
*1958 Dennis, C. B., 42 Pinner Park Avenue, Harrow, Middx
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1944 Digby, P. S. B., Dept. of Biology, St Thomas's Hospital Medical School, Westminster, London, S.W. 1
1955 Dimelow, Miss E. J., Dept. of Biology, Mount Allison University, Sackville, New Brunswick, Canada
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1956 LeSueur, R. F., The Museum, Jersey, C.I.
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1951 Lightowlers, F., 60 Harberton Park, Belfast, N. Ireland
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1932 Lisbon: Instituto de Biologia Maritima, Cais do Sodré, Lisbon, Portugal
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1950 Lowenstein, Prof. O. E., D.Sc., Ph.D., F.R.S., Dept. of Zoology & Comparative Physiology, The University, Edgbaston, Birmingham 15. (**Council**, 1952–55)
1950 Lowy, J., Ph.D., Biophysics Research Unit, King's College, Strand, London, W.C. 2
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1956 Lunz: Biologische Station Lunz, Lunz am See, Niederösterreich, Austria
1953 Lynch, Dr G. A. C., Beeches, Crapstone, Yelverton, Devon

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*1925 Magdalen College, Oxford
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1939 Needham, Joseph, Sc.D., F.R.S., Gonville and Caius College, Cambridge
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1910 PLYMOUTH EDUCATION AUTHORITY, Cobourg Street, Plymouth, Devon
1906 PLYMOUTH, PORT OF, INCORPORATED CHAMBER OF COMMERCE, Plymouth, Devon
1924 PLYMOUTH PROPRIETARY LIBRARY, 1 Alton Terrace, North Hill, Plymouth, Devon
1929 PLYMOUTH PUBLIC LIBRARY, Tavistock Road, Plymouth, Devon
1948 POLLARD, D. G., 60 Alcester Street, Stoke, Plymouth, Devon
1950 POLYTECHNIC, The Library, Regent Street, London, W. 1
1947 PORTSMOUTH GRAMMAR SCHOOL, Biology Dept., Portsmouth, Hants
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INDEX

GENERA AND SPECIES

Abralia, 745
Acanthephyra multispina, 735–7, 746
— *purpurea*, 711–13, 722, 735, 742–4
Acartia, 467
Acholoë astericola, 33–41, 547
Acrothrix gracilis, 695
Actinia, 804
— *equina*, 159, 198, 204–8
Actinoptychus senarius, 780
Actinothoë sphyrodeta, 474, 480
Adocia cinerea, 480
Aeginura grimaldii, 710–15
Aequorea, 715–16
— *forskalea*, 547
Ahnfeltia plicata, 691, 779
Akera, 295
— *bullata*, 24–5
Alaria esculenta, 159, 181–9, 198–9, 473, 479, 691
Alcyonidium, 569, 578
Alcyonium digitatum, 474, 480–1, 611
Ammodytes, 825
Ampelisca brevicornis, 97
Amphilectus fucorum, 474
Amphinema krampi, 81–4, 821
Amphioxus, 117–26, 813
Amphipleura rutilans, 780
Amphritrite johnstoni, 16–22, 25–6, 28–9
Amphora arenicola, 780
— *exigua*, 780
Anapagurus, 832
Anemonia sulcata, 159, 166, 182–5, 198, 204–8
Anguillulina fucicola, 147
Anisopsammia rostrata, 640
Anodonta cygnea, 437
Antedon, 814
Antho involvens, 478
Antithamnion sarniense, 690–4
Aplidium nordmanni, 649–51
— *pallidum*, 649–51
— *punctatum*, 649–51
Aplysia, 28, 295
Apoglossum ruscifolium, 690
Archidistoma aggregatum, 475
Arenicola, 475
— *branchialis*, 267–85
— *claparedii*, 283
— *cristata*, 284
— *ecaudata*, 267–85
— *grubii*, 267
Arenicola marina, 17, 19, 24–9, 269, 279, 521–9
Argyropelecus olfersi, 737–41
Arion ater, 28
Aristaeus coruscans, 723
Arnoglossus laterna, 824
Artemia salina, 85–96, 830
Arthessa, 296
Arthrocladia villosa, 691
Ascophyllum nodosum, 145–7, 154–9, 181, 610, 692, 776–7, 779
Asparagopsis armata, 690
Aspergillus niger, 607
Asperococcus bullosus, 691, 696, 779
Astacus, 379
— *fluviatilis*, 389
Asterias forbesi, 571, 577, 586
— *rubens*, 28, 473, 512, 566–89, 607, 611, 813
— *vulgaris*, 587
Asterionella japonica, 397–413, 781
Asymphylodora progenetica, 454
Atolla parva, 710, 719
— *vanhöffeni*, 821
— *wyvillei*, 710–19, 741–8, 826
Audouinia tentaculata, 15–19, 23
Aulosphaera triodon, 709–15, 742–4

Bacciger bacciger, 451
Bacillus phosphorescens, 39
Balanophyllia regia, 475, 823
Balanus, 427, 633
— *amphitrite*, 502, 512
— *balanoides*, 52–63, 158–208, 432, 484–9, 508–12, 611
— *balanus*, 159, 179, 198, 432
— *corolliformis*, 635
— *crenatus*, 159, 172, 193–9, 204–8, 432, 484–5, 508–10
— *hirsutus*, 633–5
— *improvisus*, 159, 172, 198, 204–8, 484–5, 508–13
— *perforatus*, 52–63, 159–208, 509
— *porcatus*, 484
Bathytroctes rostratus, 727
Beroë ovata, 547, 711–14, 717, 719–22, 741–4, 748
Biddulphia alternans, 780
— *aurita*, 780
— *mobiliensis*, 780
Bifurcaria rotunda, 159, 181–2, 189, 190, 195, 198–9

Bifurcaria tuberculata, 185
Bispira volutacornis, 475
Bithynia tentaculata, 454
Blennius ocellaris, 450
Blidingia minima, 779
Bodianus rufus, 450
Bombyx, 829
Bonnemasonia asparagoides, 691
— *hamifera*, 691, 696
Botrylloides leachii, 474
Brachiodontes senhausi, 450–3
Brongniartella byssoides, 690–4
Bryopsis, 815
Bryssopsis lyrifra, 607
Buccinum undatum, 610–14
Bucephalopsis, 814
— *tylosuris*, 442
Bugula neritina, 502
— *turbinata*, 474

Calanus, 448–50, 537, 815
— *bajanado*, 450
— *calanus*, 450
— *finmarchicus*, 459–71
— *helgolandicus*, 460–7
Calliactis parasitica, 789–96, 805
Calliblepharis ciliata, 691
Calliostoma zizyphinum, 52–5, 159, 179, 198, 204–8
Callithamnion hookeri, 779
— *tetragonum*, 691
— *tetricum*, 779
Callocolax neglectus, 690
Callophyllis laciniata, 690
Cambarus, 745
Cancer, 388, 828
— *pagurus*, 394, 611
Candacia, 193
Carcinus, 388–95, 828
Caryophyllia smithi, 474–80, 640
Caulolatilus anomalus, 450
Centropages, 467
— *hamatus*, 196
— *typicus*, 196
Ceramium ciliatum, 775, 779
— *deslongschampsii*, 779
— *diaphanum*, 690, 775, 779
— *flabelligerum*, 779
— *rubrum*, 150, 690, 771–9
— *tenuissimum*, 691
Ceratium furca, 365–6
— *longipes*, 365
Chaetomorpha aerea, 779
— *melagonium*, 690, 696
Chaetopteris plumosa, 691
Chaetopterus variopedatus, 43, 714–15, 732, 826
Chaetozone, 16
Chauliodus, 737–9, 746
— *sloanei*, 755–65
Chiasmodon, 765
Chirostomias pliopterus, 734
Chlamys opercularis, 610–14, 665
Chlorella pyrenoidosa, 784, 819
Chondria dasyphylla, 691
Chondrus crispus, 154, 690, 771–9
Chorda filum, 689, 695, 779
Chordaria flagelliformis, 691
Chrysochromulina, 209–28, 819
— *alifera*, 211
— *brevifilum*, 211
— *chiton*, 209, 212–28
— *ephippium*, 211–14
— *ericina*, 211
— *kappa*, 211, 223
— *minor*, 211
Chthamalus stellatus, 50–63, 158–208, 427, 432, 509–13
Chylocladia verticillata, 690
Ciona, 117, 119, 122–3
Cirratulus cirratus, 16, 19, 23
Cladophera, 771–9, 816
— *rupestris*, 690
Cladostephus spongiosus, 771–9
— *verticillatus*, 691, 696
Clavelina, 480
— *lepadiformis*, 649–50
Cliona celata, 481
Clione limacina, 287
Cocconeis costata, 780
— *dirupta*, 780
— *scutellum*, 780
— — var. *stauroneiformis*, 780
Cochlodesma praetenue, 97–112
Codium, 610
— *tomentosum*, 779
Coelorhynchus, 741
Coitocaecum anaspidis, 444
Colobonema sericeum, 710, 712–13, 716
Colpomenia peregrina, 779
Conger conger, 808
Corallina officinalis, 610, 690, 771–9
Corynactis viridis, 476–81, 823
Corynospora pedicellata, 691
Corystes cassivelaunus, 611
Coscinodiscus excentricus, 780
Crangon vulgaris, 306
Crassostrea, 424
— *virginica*, 591
Crella inflata, 823
Crenilabrus melops, 453
— *pavo*, 450
Crepidula fornicata, 512, 565–89
Crisia, 477–9
Crossota alba, 710, 713, 715
Cruoria pellita, 690

Cryptopleura ramosa, 690
Ctenolabrus rupestris, 755–66
Cucumaria normani, 475
Cutleria multifida, 691
Cyclothone, 737–9, 746
— *livida*, 738, 740
Cypridina, 722, 747
— *hilgendorfi*, 722
Cystoclonium purpureum, 690, 775, 779
Cytocladus, 548
— *major*, 709–15, 742–4

Dactylocotyle gadi, 67
Dallina septigera, 640
Daphnia, 358, 368
Dasyatis pastinaca, 302
Delesseria sanguinea, 474, 690, 696
Dendrocoelum lacteum, 742
Dendrodoa grossularia, 481, 569
Dendrophyllia, 640
Dercitus bucklandi, 478
Desmarestia, 667
— *aculeata*, 689, 694–5, 700
Diadumene, 578
Diaphus rafinesquei, 756–65, 828
Diazona violacea, 478, 481
Diclidophora denticulata, 67–79
— *gadi*, 67–77
— *luscae*, 68–79
— *maccallumi*, 67
— *macruri*, 68–78
— *merlangi*, 68–77
— *minor*, 66–78
— *morrhuae*, 67
— *palmata*, 68–79
— *phycidis*, 68, 73
— *pollachii*, 68–77
Dictyopteris membranacea, 478, 691
Dictyota dichotoma, 480, 689, 690, 774–9
Didemnum candidum, 649–51
Diogenes, 832
Diploneis bombus, 780
Distomum subtenue, 448
Distomus, 479
— *variolosus*, 477
Drosophila, 242
Dudresnaya verticillata, 691
Dumontia incrassata, 691, 779
Duvaucelia, 28
Duymaeria flagellifera, 450
Dysidea fragilis, 477

Echinocardium cordatum, 97
Echinus, 424, 481
— *esculentus*, 331, 343–7, 480–1, 611, 822–3
Echiostoma tanneri, 741
Ectocarpus, 696, 814
— *confervoides*, 690
Elminius modestus, 52–5, 85–96, 159–208, 483–520, 830
Elysia viridis, 237
Ensis siliqua, 97
Enteromorpha, 231, 774–9
— *clathrata*, 690, 696
— *compressa*, 690
— *linza*, 691
Epinephelus aka-ava, 450
Eudactylina acuta, 113
— *rachelae*, 113–16
— *similis*, 113–15
Euglena gracilis, 673–84
Eunice oerstedii, 640
— *pennata*, 640
Eunicella verrucosa, 478
Euonymus, 735–6
Eupagurus bernhardus, 379–95, 789, 803
Euphausia pacifica, 39, 713, 739–44
Eurypharynx pelecanoides, 762
Eustomias bibulosus, 734
Evadne, 193
— *nordmanni*, 349–70
Exuviaella mariae-lebouriae, 820

Flabelligera affinis, 17, 19, 23, 29
Fucus, 145–55, 692, 775
— *serratus*, 146, 153–9, 181, 198, 610, 690–2, 771–9
— *spiralis*, 154, 159, 181, 198, 610, 776–9
— *vesiculosus*, 145–59, 181, 198, 610, 690, 771–9
Furcellaria fastigiata, 154, 691, 771, 779

Gadus aeglefinus, 68, 77
— *luscus*, 68, 302–3
— *merlangi*, 68
— *minutus*, 756, 765
— *morrhua*, 67
— *pollachius*, 68, 302
— *poutassou*, 68, 78
— *virens*, 68
Gastroclonium ovatum, 779
Gastrocotyle trachuri, 446
Gastropteron, 296
Gelidium coinale, 779
— *latifolium*, 690, 779
Gibbula, 578
— *cineraria*, 52–5, 159, 178, 198–208
— *magus*, 159, 179–98
— *pennanti*, 159–208
— *umbilicalis*, 52–63, 159–208, 611
Gigartina stellata, 610, 690, 779
Glenodinium foliaceum, 324–7
Gnathia maxillaris, 305
— *oxyurea*, 305
Gobius minutus, 306
Goniaulax, 364 (see *Gonyaulax*)

Gonospora arenicolae, 277
— *minchini*, 278
Gonostoma bathyphilum, 756
— *denudatum*, 738–40, 756–66
— *elongatum*, 754–66
Gonyaulax, 544–7
— *polyedra*, 545–7, 741
Gracilaria verrucosa, 691, 694
Grammatophora oceanica, 780
Griffithsia flosculosa, 691, 779
Gymnodinium veneficum, 820

Halarachnion ligulatum, 691
Halenchus, 145–55
— *dumnonicus*, 145–54
— *fucicola*, 145–54
— *mediterraneus*, 153
— *mexicanus*, 153
Halichondria panicea, 477, 481, 611
Haliclona, 823
Haliclystus, 822
Halidrys siliquosa, 690, 779
Halimeda gracilis, 610
Haliotis tuberculata, 159, 179, 182, 198
Haliphysema tumanowiczi, 474
Halopitys incurvus, 779
Halopteris filicina, 478
— *scoparia*, 779
Helix, 808
Hemimycale columella, 474–80
Hemioniscus balani, 159, 167–208
Hemiselmis virescens, 324–7
Henricia sanguinolenta, 611
Heterocarpus alphonsi, 723
Heterosiphonia, 475
— *plumosa*, 691
Heteroteuthis, 744
Hexelasma, 824
— *americanum*, 635
— *corolliforme*, 635–6
— *hirsutum*, 633
Himanthalia elongata, 159, 181–99
Hippopodius hippopus, 710, 712–13, 717
Hispanirhynchia cornea, 635, 640
Holmsella pachyderma, 691
Holothuria forskali, 475, 481, 832
Homarus vulgaris, 299, 379–94, 603–6
Hoplophorus, 739
— *grimaldii*, 745
— *novae-zealandiae*, 723
— *spinicauda*, 723
Hydrurus, 224
Hymedesmia, 479, 640
Hypoglossum woodwardii, 690
Hypsechinus, 640

Ianthina janthina, 5–8, 821
— *planispirata*, 5
Idiacanthus, 761
Idotea, 693, 815
Inidio buvittata, 450
Isochrysis, 346
— *galbana*, 416–17, 535, 781–7
Isthmoplea sphaerophora, 691

Jania rubens, 779
Julis pavo, 450

Korethraster hispidus, 640
Krampella dubia, 83–4, 821
Kuhnia, 74, 77
— *scombri*, 67

Labrus bergylta, 302, 452, 758
— *merula*, 450
— *ossifagus*, 304–6
Lachnolaimus maximus, 450
Lactobacillus leichmannii, 678–9, 684
Lagisca extenuata, 33–41
Lamellisabella zachsi, 628
Laminaria, 474–80, 607, 689–97, 775
— *digitata*, 159, 180, 198, 610, 689–92, 776–9, 825
— *hyperborea*, 159, 180, 198, 473–9, 689–92
— *ochroleuca*, 159, 180–2, 198
— *saccharina*, 159, 180, 198, 667–8, 689–99, 701–2, 779
Lanice conchilega, 16, 19, 23, 97
Lasaea rubra, 107, 815
Latridopsis ciliaris, 450
Laurencia hybrida, 774–9
— *pinnatifida*, 771–9
Leachia, 745
Leander serratus, 85–96, 829–30
Leathesia difformis, 775–9
Lemna minor, 607
Leoptilus, 554
Lepidochitona cinerea, 237
Lepomis, 742
Leptosomias bermudensis, 734
— *gladiator*, 734
— *macropogon*, 734
— species, 734
Leuconia nivea, 477
Leucosolenia complicata, 832
— *coriacea*, 478
Licmophora gracilis, 780
— *lyngbei*, 780
Lima, 823
— *hians*, 480
Limacina retroversa, 289
Lissoclinum argyllense, 649–51
Lithodes, 832
Lithophyllum, 610
Lithothamnion, 480, 610, 690, 774–9
Litosiphon pusillus, 690

Littorina exigua, 248, 259
— *littoralis*, 198, 611
— *littorea*, 57, 159, 178–208, 229–66, 512, 611
— *neritoides*, 56, 159–208, 262
— *obtusata*, 56–7, 159, 204–8, 262
— *rudis*, 259
— *saxatilis*, 56–7, 159–60, 178, 198–208
Loligo forbesi, 306
Lomentaria articulata, 690, 771–9
Lophius, 829
Lophohelia, 640
Lucernariopsis campanulata, 822
— species, 822
Luidia ciliaris, 611
Lyocopodium flabelliforme, 607
Lyonsia, 98, 100

Macoma balthica, 436
Macruris rupestris, 68, 78
Magelona alleni, 617–26
— *cincta*, 617–26
— *japonica*, 620
— *longicornis*, 620
— *obockensis*, 620
— *papillicornis*, 617
— *rosea*, 620
Maia, 394, 807, 816, 828–31
Malacocephalus, 732
— *laevis*, 741
Marthasterias glacialis, 611
Mastogloia binotata, 780
Melinna palmata, 17–19, 23–5
Membranoptera altata, 690, 774, 779
Merga rubra, 81
Merluccius merluccius, 755
Metridium dianthus, 474
— *senile*, 789–809, 832
Microglena arenicola, 324–7
Microstomus kitt, 302
Milio macrocephalus, 450
Mitchella repens, 607
Mitrocoma, 715
Mnemiopsis leidyi, 39, 547, 713–21, 739–44
Moina, 361
Molgula manhattensis, 808
Molva molva, 68, 78
Monocentris, 732
Monochrysis lutheri, 324–7, 674
Monodonta lineata, 52–63, 159, 175–208
Munida microphthalma, 640
— *tenuimana* 640
Mya, 101
— *arenaria*, 436
Mycale macilenta, 478
Myctophum punctatum, 711–13, 726–48
Myrionema strangulans, 691
Mytilus, 192, 473, 575, 797, 808
— *edulis*, 199, 611

Myxicola infundibulum, 17, 19, 24, 815
Myxilla rosacea, 477–9

Naccaria wiggii, 691
Nannochloris oculata, 324–7
Navicula cluthensis, 780
— *cyprinus*, 780
— *distans*, 780
— *grevillei*, 780
— *mollis*, 780
— *ramosissima*, 780
Nemertesia, 480
Nephthys hombergi, 97
Nereis, 814
Nitophyllum punctatum, 691
Nitzschia acuminata, 780
— *angularis*, 780
— *closterium*, 607
— — forma *minutissima*, 324, 781
— *lanceolata*, 780
— *panduriformis*, 780
— *punctata*, 780
— — var. *coarctata*, 780
— *sigma* var. *rigidula*, 780
Noctiluca, 535–549, 826
— *miliaris*, 39, 535, 547
Nucella lapillus, 512
Nyctiphanes couchi, 746

Obelia, 804
Ochrosphaera neapolitana, 224
Octophialucium funeraria, 715
Octopus vulgaris, 195–6, 831
Odonthalia dentata, 690
Onchidium, 237
Ophiacantha, 640
Ophiactis, 640
Ophiocomina nigra, 611
Ophiothrix fragilis, 474
Ostrea edulis, 415–25, 512, 591–602
— *gigas*, 450–2
Otina otis, 295
Owenia fusiformis, 521–9
Oxynoë, 296
Oxyrrhis marina, 324–7

Pachygrapsus marmoratus, 182, 195
Pachymatisma johnstoni, 478
Pagellus centrodontus, 299, 302
Pagrosomus auratus, 450
Pagurus, 832
Palinurus vulgaris, 299, 302, 379, 388–94
Pandalus, 829
Pandora, 98–110
Paracentrotus lividus, 159, 176–208
Parajassa pelagica, 477–9
Paralepis rissoi, 764
Paralepoderma brumpti, 454

Paralia sulcata, 780
Paravortex scrobiculariae, 439
Patella, 237
— *aspera*, 56, 159, 175–208
— *depressa*, 56, 159, 174–208
— *intermedia*, 174, 182, 190
— *vulgata*, 56, 159, 174–208, 263, 611
Pecten maximus, 610–14, 635–71, 815
Pectinaria, 15, 17
Pelonaia corrugata, 650–1
Pelta, 814
Pelvetia canaliculata, 159, 181, 198, 610, 692
Pennatula phosphorea, 551–63
Peridinium, 364–7
— *triqueta*, 367
— *trochoideum*, 324–7
Periphylla periphylla, 719
Petalonia fascia, 779
Petricola pholadiformis, 452
Phacoides borealis, 97
Phaeocystis, 363
Phaeodactylum, 416
— *tricornutum*, 324–7, 607, 781, 819
Phallusia mammillata, 478
Phascolion, 814
Pholas dactylus, 43–7, 826
Phoronis hippocrepia, 475, 823
Photoblepharon palpebratus, 739
Phycodrys rubens, 690
Phyllophora membranifolia, 690, 779
Pinna, 103
Platidia, 640
Plectanocotyle, 71–7
— *gurnardi*, 67, 71
Plesiopenaeus coruscans, 723
Pleuronectes platessa, 302, 309
Plocamium coccineum, 689, 690, 779
Plumaria elegans, 690
— *setacea*, 477
Podon, 193
Podosira stelliger, 780
Pollicipes cornucopiae, 182
Polycarpa pomaria, 477
Polycirrus caliendrum, 17, 19, 24
Polyclinum aurantium, 649–51
Polydora ciliata, 512
Polyides caprinus, 691, 779
Polymnia nebulosa, 15
Polysiphonia, 690–1, 696
— *denudata*, 779
— *elongata*, 690
— *fibrata*, 780
— *lanosa*, 610, 780
— *nigra*, 691
— *nigrescens*, 690, 771–4, 780
— *spiralis*, 691
— *urceolata*, 690, 780
— *violacea*, 690
Pomatoceros, 475, 823
— *triqueter*, 325–22
Porania pulvillus, 610–4
Porichthys, 832
Porphyra umbilicalis, 691, 780
Proctoeces erythraeus, 439–55
— *insolitus*, 447–55
— *macrovitellus*, 447–55
— *maculatus*, 447–50
— *magnorus*, 447–55
— *major*, 447–50
— *ostreae*, 441–53
— *subtenuis*, 435–56
Prorocentrum, 815
Protula tubularia, 475
Prymnesium parvum, 323–7, 781, 820
Psammechinus, 816
Psolus squamatus, 640–3
Pterosiphonia complanata, 690
— *thuyoides*, 780
Ptilota plumosa, 690, 771, 780
Pylaiella littoralis, 779
Pyrgoma anglicum, 474
Pyrosoma, 745, 748, 826
— *atlanticum*, 39, 547, 711–44
— *elegans*, 724
— *spinosum*, 723–6

Radopholus, 153
Raia, 302
Ramulina, 611
Raspaciona aculeata, 823
Raspailia hispida, 475
Ratzia joyeuxi, 454
Renilla köllikeri, 547, 554, 562
Rhabdonema arcuatum, 780
— *minutum*, 780
Rhabdostyla arenicolae, 274
Rhaphoneis amphiceros, 780
— *surirella*, 780
Rhodochorton floridulum, 771, 780
Rhodomela confervoides, 690, 696, 701, 780
Rhodymenia palmata, 154, 610, 690, 771–80
Rhoicosphenia curvata, 780
Rhombognathus (*Rhombognathopsis*) *seahami*, 148
Rhopalodia musculus, 780
Rhynconella sicula, 635
Rosacea plicata, 710–17

Sabellaria, 565–9
— *alveolata*, 185, 343
— *spinulosa*, 185
Saccopharynx, 764
Saccorhiza, 473
— *bulbosa*, 480
— *polyschides*, 159, 180–98, 689–702
Sagartia elegans, 477

Saggita elegans, 193
— *setosa*, 193, 821
Sardina pilchardus, 197
Sciurus niger, 28
Scomber scombrus, 302, 754
Scopelus, 733
Scrobicularia plana, 101, 435–56, 814
Scrupocellaria reptans, 477
Scytosiphon lomentaria, 224, 691
Searsia, 763
— *koefoedi*, 711–13, 726–8, 742
— *rostratus*, 727
— *schnakenbecki*, 711, 726–32, 748
Seirospora griffithsiana, 691
Semicossyphus reticulatus, 450
Sergestes, 745
Sertularia, 569
— *operculata*, 477–80
Siboglinum atlanticum, 627–32
— *caulleryi*, 630
— *ekmani*, 627–32
— *inerme*, 627–32
— *plumosum*, 630
— *weberi*, 627–8
Sidnyum turbinatum, 649–51
Skeletonema, 369
— *costatum*, 324–7
Solaster, 566–79
— *endeca*, 569
— *papposus*, 565
Sorocarpus micromorus, 690
Spanioplon armaturum, 479
Sparus australis, 450
— *bifasciatus*, 450
— *sarba*, 450
Spatangus purpureus, 611
Spermothamnion barbatum, 780
— *repens*, 691
Sphacelaria fusca, 779
— *pennata*, 690
— *radicans*, 779
Sphondylothamnion multifidum, 691
Spondyliosoma cantharus, 299–304
Spongonema tomentosum, 691
Sporochnus pedunculatus, 691, 694
Spyridia filamentosa, 780
Squalus acanthias, 607
Stichastrella rosea, 611
Stictyosiphon subarticulatus, 690
Stelligera stuposa, 475
Stereocidaris ingolfiana, 640
Stolonica socialis, 474–7
Suberites domuncula, 474
— *epiphytum*, 477
Symphodus griseus, 450
Synedra affinis, 780
Synedra gaillonii, 780
— *investiens*, 780
— *tabulata*, 780
Syracosphaera carterae, 781
— *elongata*, 324–7
Systellaspis debilis, 723, 735–9, 745

Talitrus saltator, 264
Tellina fabula, 97
— *tenuis*, 823
Temora, 467
Terebella lapidaria, 16, 19, 23, 29, 809, 832
— *meckelii*, 15
Tethya aurantium, 475
Thalassiosira decipiens, 780
Thalassoma lunare, 450
Thaumatolampas diadema, 741
Thoracophelia mucronata, 23
Thracia, 100–1
Tilopteris mertensii, 691
Tintinnopsis, 365
Tomopteris erythraea, 439
Torpedo, 830
— *nobiliana*, 113–16
Trachurus trachurus, 446, 824
Trachyneis aspera, 780
Trigla cuculus, 824
Tubipora, 561
Tylenchus fucicola, 145, 147
— *zostericola*, 53

Ulosa tupha, 474
Ulothrix flacca, 774–9
Ulva lactuca, 231, 248, 690, 696, 771–9
Urophycis blennioides, 68, 78
Urosalpinx, 511–19

Venus mercenaria, 669
— *striatula*, 97
Verruca coraliophila, 638–9
— *halotheca*, 639
— *linearis*, 637
— *magna*, 638
— *mirabilis*, 551–63
— *recta*, 633
— *stroemia*, 159, 173–208, 427–33, 481
Vogtia glabra, 710–17, 741, 748
— *spinosa*, 710–16, 742–4

Xenodermichthys copei, 737–9, 754–6, 765–6

Zeus faber, 306
Zirphaea crispata, 107
Zostera, 153
— *nana*, 779